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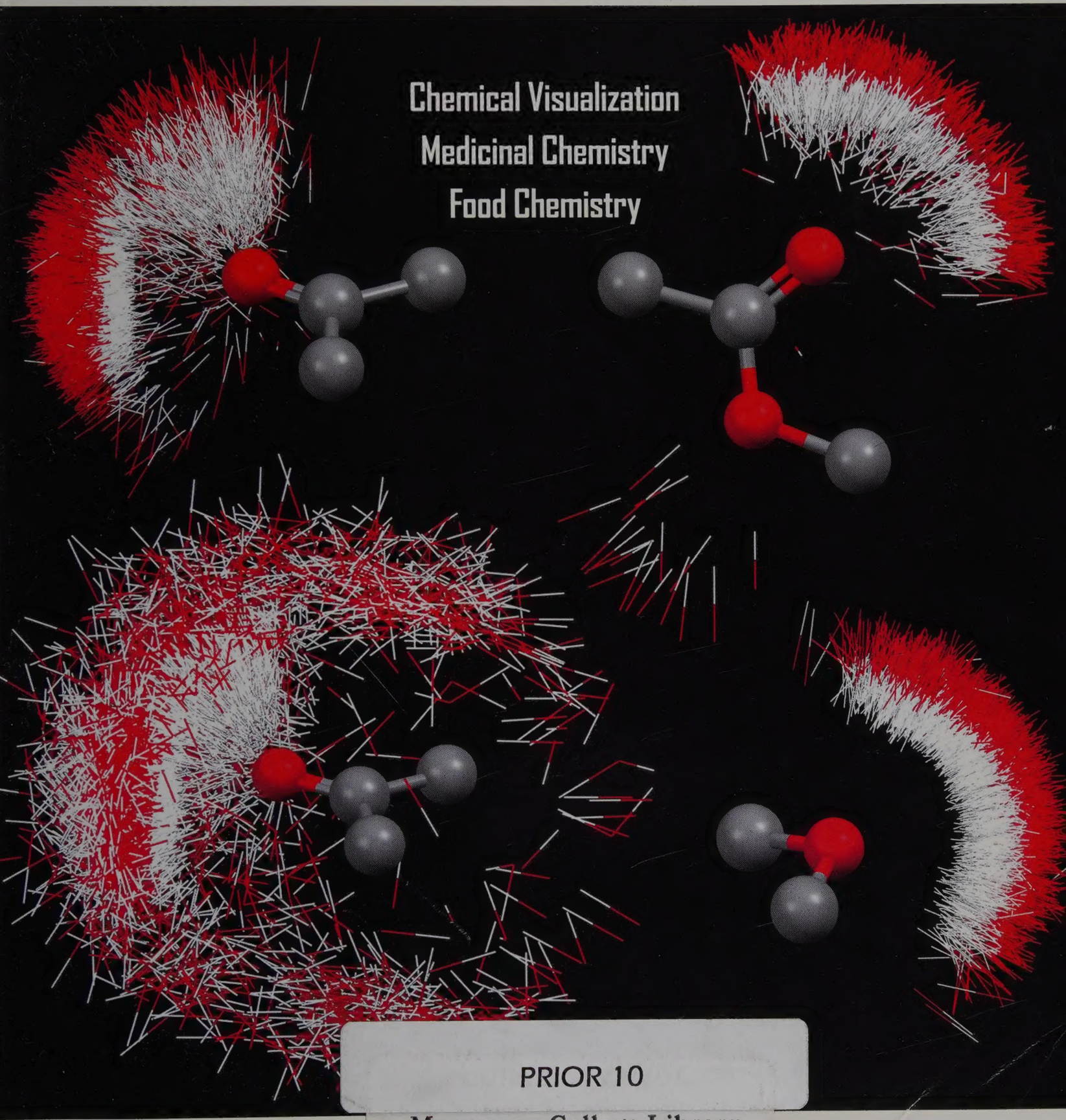

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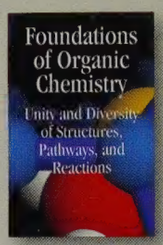

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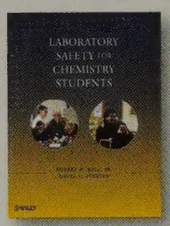

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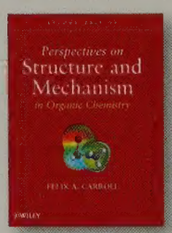

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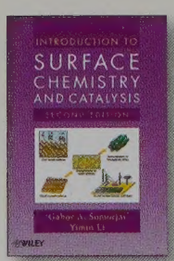

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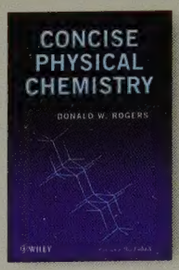

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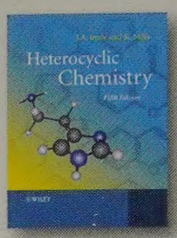

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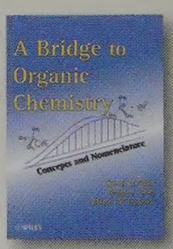

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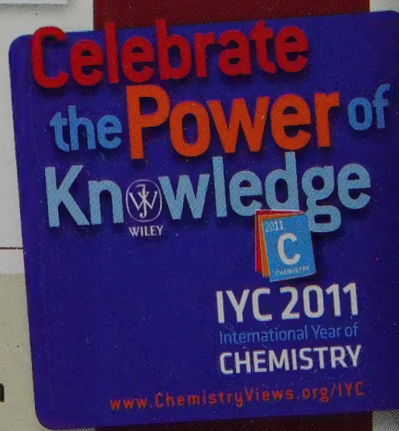

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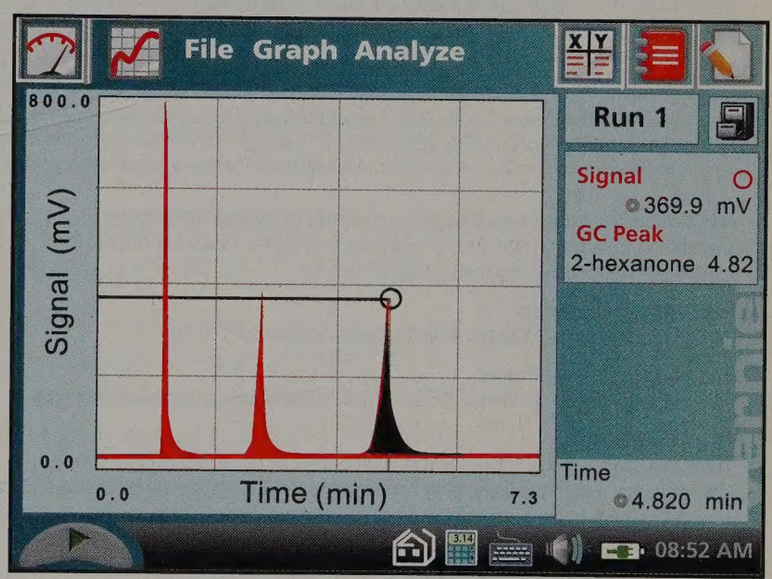

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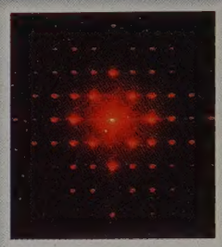
- **Discovery Slide:** provides multiple examples for changes in orientation and in unit cell size; good for student investigations
- **VSEPR Slide:** complements discussions of rules for predicting molecular geometry and illustrates how geometry differences can be detected in a triatomic molecule
- **Plane Groups Slide:** provides additional examples for the systematic absences that occur for centering and for glide symmetry.



Shining a red laser pointer through the array at the left produces this diffraction pattern.

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DNA Optical Transform Kit

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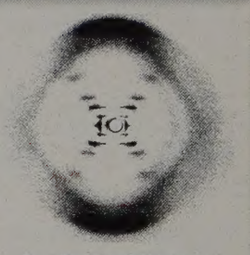
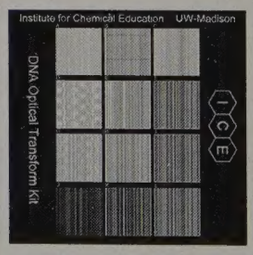


Rosalind Franklin's X-ray diffraction pattern of B-DNA.



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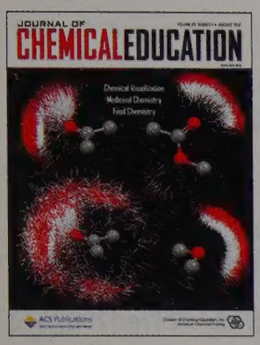

ON THE COVER: A clear understanding and appreciation of noncovalent interactions, especially hydrogen bonding, are vitally important to students of chemistry and the life sciences, including biochemistry, molecular biology, pharmacology, and medicine. In the article "Learning about Intermolecular Interactions from the Cambridge Structural Database" (DOI: 10.1021/ed200139t), Gary M. Battle and Frank H. Allen discuss the opportunities afforded by the IsoStar knowledge base of intermolecular interactions to enhance the educational experience of students, particularly through visualization. Derived from experimentally determined crystal structures in the Cambridge Structural Database and Protein Data Bank, IsoStar contains geometrical and spatial information on nearly 30,000 different types of interactions in small molecules and proteins. See the article for detailed descriptions of the example IsoStar scatterplots shown on the cover.

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EDITORIAL

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Declaring a New Year's Resolution

Norbert J. Pienta*

Department of Chemistry, University of Iowa, Iowa City, Iowa 52242-1294 United States

ABSTRACT: Innovations and changes in courses are discussed and readers are encouraged to adopt evidence-based standards in their decision making.

KEYWORDS: General Public, Curriculum

The time just before the beginning of a calendar year is a traditional opportunity to make personal changes, so-called New Year's resolutions. Typically, these commitments are ones that individuals make about personal habits or lifestyle changes. Although it might seem timely for this author to declare that he will endeavor to write better commentaries while simultaneously implementing more healthful eating habits, this missive concerns a professional perspective. That is probably good news because in late 2009, the *Wall Street Journal* reported on research (based on 3000 surveys) that showed that 88% of these resolutions are failures.[1] The article cites several examples of scientific research concerning the prefrontal cortex of the brain and its rather limited control over willpower. The most significant piece of advice from that article is to be self-aware about the shortcoming that is the basis for the resolution. As a professional courtesy and implicit with my responsibilities as Editor, this editorial provides a New Year's resolution for the chemical education community.

A late fall visit to a large southwestern university afforded the author exposure to a set of modern educational developments, apropos to the digital age. Consistent with a large and diverse student population and with distances involved in an urban setting, science classes, including chemistry, are being developed and implemented in the online and distance-learning categories. This is not meant to be news; many institutions have contributed in this arena. Rather this educator was struck by several aspects of the process, certainly not something unique to the school being visited. The issues are how educators and administrators that represent them have implemented the changes. Converting a "live" lecture to one on streaming video can have advantages: the material can be viewed more than once and at a time when the viewer is more receptive. (Personally, my preference would be 7:30 p.m. rather than 7:30 a.m., but my point is about having the choice.) Of course, if the instructor is just reading from some slides, converting the lecture to a digital form will not be any more effective as a video. That is not to say that there is no value to lecturing. Used appropriately and *as part of a more comprehensive plan*, those didactic portions can help organize and clarify the information for students to assimilate.

My concern here has to do with how teaching practices correspond with how students learn. For example, we know that active methods are better than passive ones, that knowledge is built, not simply transferred, and that certain cyclical models are especially effective.[2] This understanding about learning is evidence-based, with most or all of the information coming not from experiences but carefully derived from experiments.[2] While experience can be a useful guide, one must be careful not to confuse longevity with excellence. Having survived for 30 or 40 years does not mean that a practice is the best or even very effective. It could simply be that it was the only reasonable or cost-effective way to do something in the past. Think about the humble yet useful glass buret. Would it be the best method to accurately measure amounts of solutions today? Or is our thinking simply guided by history and tradition? Recall that many generations of users of balances concerned themselves with counting swings of arms and chains.[3] This time has passed and so will other familiar habits.

Our community will continue to be confronted with change, including methodologies that are novel. Science is conservative, seeking clear evidence before a change becomes part of the commonly accepted standard. Chemical education also behaves in this way. But the operative concern should be effectiveness, not that a method or idea is new. The beginning of the 89th volume of the *Journal* provides opportunities for self-reflection and adjustment. Make a New Year's resolution to look at what you are currently doing with your teaching. Endeavor to make it the best you can from the perspective of student learning. Make that the goal, keeping in mind those ideas that are evidence-based. Even with only a 12% success rate, chemical education could be truly transformed in under a decade. And with a commitment to such a lofty resolution, have another holiday cookie.

AUTHOR INFORMATION

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*Corresponding Author E-mail: norbert-pienta@jce.acs.org.

REFERENCES

(1) Lehrer, J. Blame It on the Brain. *The Wall Street Journal*, Dec 26, 2009; http://online.wsj.com/article/SB10001424052748703478704574612052322122442.html (accessed Nov **2011**).

(2) Two sets of monographs that summarize many aspects of learning in the language of chemistry can be found in *Chemists' Guide to Effective Learning* (Vols. 1, 2), Pienta, N. J., Cooper, M. M., Greenbowe, T. L., Eds.; Prentice-Hall: Upper Saddle River, NJ, 2005 and 2009.

(3) Lewin, S. Z. Balances. *J. Chem. Educ.* **1959**, *36* (1), A7−A14; DOI: 10.1021/ed036pA7.

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COMMENTARY

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Become a *Journal of Chemical Education* Author

Erica K. Jacobsen*

The Dalles, Oregon 97058, United States

Supporting Information

ABSTRACT: Preparing and submitting a manuscript to a professional journal can be a daunting task, particularly for high school educators. The author discusses reasons one might choose to write for the *Journal of Chemical Education*, identifies potential obstacles in preparing and submitting a manuscript, suggests ways to overcome these obstacles, and encourages educators to take the next step toward becoming a *JCE* author.

KEYWORDS: High School/Introductory Chemistry, Professional Development

I don't have time.
I can't write.
I don't have any worthy ideas.
I don't have enough knowledge.
It won't be accepted anyway.
I don't want to undergo peer review.
It's scary!

The list above shares the most common answers to the question "What is your biggest obstacle to publishing your ideas?" from a brief survey I have given at the start of a workshop presentation titled, Publishing Your Chemical Education Ideas: What, How, When, Where, Why? In presenting to several different groups of high school and college-level educators, the same answers arise every time.

During my time as part of the precollege chemistry section of the *Journal of Chemical Education* (*JCE*), a constant goal of the section has been to encourage more high school educators to submit their work to *JCE*. Working toward that goal has included the workshop presentation mentioned above, speaking to potential high school authors, following up with those authors, and providing constructive feedback on ideas and early drafts. Progress has been slow, yet encouraging. Over the past nine years, numbers for a yearly total of high school authors have risen from the low teens to the low twenties. However, given that a recent single issue of *JCE* had over 60 authors, there is room for improvement.

WHY WRITE?

The obstacles listed above can be formidable and the section below suggests how each can be overcome. However, even before that, the first question to ask is: Why do you wish to publish your chemical education ideas? During presentations, certain answers to this question crop up again and again. Those at the college level sometimes bring up the phrase "publish or perish". For high school educators, it is quite rare to encounter this pressure. Authoring a publication in a professional journal, particularly one recognized as being a major journal in its field, can be a good résumé builder at the high school level. Preparing an idea for publication can also be a good opportunity for professional growth. It can help even a seasoned educator to review and clarify what they know about chemistry and teaching. Other important reasons are that it is a way to share useful curriculum with a wider audience and to give back to the educational community, even beyond the time of one's teaching career. Some articles that were published long ago in *JCE* still find use today. A high school teacher who recently published an article in *JCE*, Peter Heid, provides an additional reason for pursuing publication: "I thought that I could also demonstrate (model) setting a goal and achieving a goal to my classes, so off I went."[1]

OVERCOMING OBSTACLES

Time

High school educators in particular have many school-related demands on their time, in addition to the regular flow of everyday life. Many books have been written on time management techniques, but there really is no silver bullet. If you decide that writing an article is important and a priority for you, you will make the time. One potential pitfall is to view writing an article as a single, major event; it seems as though it will take such a long amount of time that we never even begin. If pictured as a series of small chunks of work, it may be easier to find that shorter amount of time and only focus on the next action, whether it be beginning a literature search, selecting keywords, writing an introduction, creating a table, and so on.

Writing Ability

Even experienced writers may doubt their ability when faced with a new piece. One way to overcome your doubts is to ask for feedback from a friend or colleague; this could be someone in the field of chemistry education or even outside of it. Explaining your idea to someone you feel comfortable with can help you to clarify the idea within your own mind and lead to constructive questions, feedback, and, most of all, encouragement. Or, if the article is describing work done with a colleague, enlist that person as a coauthor. You can then share in the writing and bounce ideas and drafts off of each other. Understanding more about the writing process can also be helpful. I have always felt more secure in getting the first words down on the page when thinking of Anne Lamott's idea in her book *Bird by Bird*, that the first draft will be

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terrible.[2] She says, "All good writers write them. This is how they end up with good second drafts and terrific third drafts."[2]

Worthy Ideas and Knowledge

When first considering what you might write an article about, nothing may come to mind. I typically tell potential authors that everyone has an idea that could be made into an article. You can ask yourself questions such as: What particularly interests me in the classroom and my own journal reading? What ideas from others have I tweaked to "build a better mousetrap"? What have I presented at conferences? What do I most often talk to other teachers about in conversations at conferences? A review of the past year's curriculum can yield ideas. *JCE* is also looking for ideas for commentaries on issues relevant to the high school educator community. High school teachers who have published in *JCE* offer advice in this area. Paul Matsumoto suggests working in a lab during the summer, stating, "Such an experience may show a teacher the interdisciplinary/collaborative nature of science, which could lead to altering a teacher's classroom activity that leads to a publication."[3] He also encourages being open to student ideas: "Encourage your students to offer their ideas—to do it 'their way' and do not discourage your students from providing their ideas on how to solve a problem or explain something. Such an attitude may lead to a publication."[3] Recently published author Alice Putti described her experience at the Target Inquiry Program: "As part of the program we were required to submit a manuscript to a science journal. Prior to joining the program it never occurred to me to try and publish my work."[4] She also benefited from having the instructors of the program to serve as mentors who could offer encouragement.

Peer Review and Acceptance for Publication

It can be difficult, after submitting a lovingly and carefully crafted article that you feel is already perfect, to read reviews that suggest changes should be made. Brett Criswell states, "Do not take reviewers' comments personally. Everyone has a different lens with which to view intellectual materials, such as a submission, and many comments are just a reflection of different lenses."[5] The simple fact is that pretty much every manuscript gets revised at some stage. Reviewers often raise questions or make suggestions that the author may not have considered, that, if addressed in the paper, would make it even better. An ideal reviewer encourages the author by pointing out the strengths of a manuscript, yet also shares constructive criticism to improve the paper. An estimate is that *JCE* accepts and publishes roughly half of the submissions that it receives. If an author receives a decision letter that asks for revisions, the submission has definite potential if the reviews are carefully considered and used to guide a revised manuscript. At the same time, if a particular detail is brought up by a reviewer that you, as an author, feel there is a compelling reason to not change that portion of the manuscript, you can always state this reasoning in a document that lists how you addressed reviewer comments when you return your revision.

■ WHAT NEXT?

If you have decided you want to submit an article and are prepared to overcome obstacles that may lie ahead, what next? The *JCE* Web site offers resources and information to get authors started. A Web page on submissions and review offers links to information for authors, including author guidelines: http://pubs.acs.org/page/jceda8/submission/index.html (accessed Nov 2011). It also provides links to information for reviewers, which can be useful to new authors to see what peer reviewers are meant to use as criteria. Serving as a reviewer yourself can also help you to see the submission process from that viewpoint. Literature searches can be performed from the *JCE* home page at http://pubs.acs.org/journal/jceda8 (accessed Nov 2011). Mary Harris states, "Make sure your idea has not been published before or use the search to mention similar ideas that have been published in the past. Give credit where credit is due."[6] Simply reading *JCE* helps an author to know the usual style of articles, laboratory experiments, demonstrations, and so on, to know the audience they are writing for, and can even give you ideas for submissions if you have an addition or improvement on an idea shared in a previous article.

What next? Write away, write now!

■ ASSOCIATED CONTENT

Supporting Information

A list of advice and tips from several past high school educator *JCE* authors. This material is available via the Internet at http://pubs.acs.org.

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JOURNAL OF CHEMICAL EDUCATION

REPORT

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Recent Trends in Chemistry Instrumentation Requests by Undergraduate Institutions to NSF's RUI Program

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ABSTRACT: The type of chemistry instruments funded as part of the Research in Undergraduate Institutions (RUI) Program at the National Science Foundation is summarized for proposals funded in 2006−2010. The data provided report on the success rate of RUI proposals, the average size of awards, the average duration of awards, and the percentage of the budget allocated to instruments. Information is given both for new and for experienced principle investigators.

KEYWORDS: First-Year Undergraduate/General, Second-Year Undergraduate, Upper-Division Undergraduate, Laboratory Instruction, Laboratory Equipment/Apparatus

FEATURE: Instrumentation Topics for the Teaching Laboratory

In recent articles in this *Journal*, a five-year overview from 2005−2009 was given of proposals that requested an instrument for use in teaching undergraduates and were submitted by Departments of Chemistry to the National Science Foundation's (NSF) Course, Curriculum, and Laboratory Improvement (CCLI) Program,[1] and the Major Research Instrument (MRI) Program.[2] Analysis of the MRI Program focused on proposals submitted from faculty teaching at undergraduate institutions. Research-active faculty at predominantly undergraduate institutions (PUIs) also seek research support from the Research in Undergraduate Institutions (RUI) Program and often the proposal includes funds to purchase instrumentation. Frequently, these instruments are intended to do double duty serving both the instructional laboratories and the research laboratory of the principle investigator, or PI. In this report, we will give an overview of the RUI Program, a six-year summary (2005−2010) of the type of chemistry instruments funded by RUI, and the success rate of RUI proposals submitted to NSF from undergraduate institutions.

■ HISTORY OF THE RUI PROGRAM

In 1982, the Council on Undergraduate Research (CUR) proposed to the National Science Board, the governing body of NSF and policy advisors to the President and to Congress, that the RUI Program be established by Congress.[3] One year later, RUI came into existence and in 1985 the *CUR Newsletter*[4] published a description of the new RUI program. The current RUI solicitation is NSF 00-144 and it replaced NSF 94-79. Thus, the expectations of the RUI program have remained relative consistent compared to other NSF programs. However, faculty do need to remain aware of changes in the NSF Proposal and Award Policies and Procedures Guide so that critical items are not neglected in the RUI proposal. For example, in 2010 the requirement was added for a postdoctoral mentoring plan if the proposal requested support for a postdoctoral fellow, and in 2011 a Data Management Plan needed to be included in all NSF proposals.

Similar to the MRI Program, the RUI proposals are submitted to discipline-specific divisions at NSF. Faculty members in departments of chemistry and biochemistry submit proposals to the Chemistry Division (CHE), the Division of Material Research (DMR), and the Molecular and Cellular Biosciences Division (MCB). Therefore, this analysis includes RUI awards made to chemists by all three divisions. Because the operations of different disciplines may vary, it is advisable for potential PIs to contact the appropriate NSF program officer regarding expectations of the RUI program.

■ GENERAL INFORMATION ABOUT THE RUI PROGRAM, 2005−2010

Table 1 shows for the past six years the average dollar amount for all RUI awards, the average award if equipment was requested, the average amount requested for equipment, the average number of PIs, the duration, and the number of awards distributed among the different NSF divisions that support chemistry faculty. Awards that included equipment requests in the budget (Budget Line D—Equipment, where each item with a cost exceeding $5000 must be listed) are identified as awards with instruments. During the past six years, there have been 204 awards to chemistry faculty, with 78% of those funded by the NSF Chemistry Division. Of the 204 RUI awards, 127 (62.2%) awards requesting equipment were granted. The average dollar requested for equipment was $30,962 per award that made a request, and the largest budget for equipment was $103,530. Several awards had requests for equipment in the $80,000−95,000 range; however, many more awards requested equipment with a cost of $20,000 ± 5000. It does appear that over the past six years the number of RUI awards each year has been steadily

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Table 1. Summary of RUI Awards to Faculty in Chemistry and Biochemistry Departments[a]

Fiscal Year	Average for All Awards, USD	Average for Awards with Inst, USD	Average Inst Award, USD	Average PIs/ Co-PIs, *N*	Average Duration (months)	CHE Awards, *N*	DMR Awards, *N*	MCB awards, *N*	Total Awards (with Instrument)
2005	275,717	284,663	36,058	1.35	48.8	22	0	2	24 (19)
2006	243,238	275,391	22,559	1.25	48.1	22	1	2	25 (14)
2007	267,792	282,544	26,755	1.20	44.7	24	2	8	34 (20)
2008	295,873	299,452	42,800	1.35	35.5	26	5	6	37 (22)
2009	250,947	227,751	23,714	1.20	31.9	38	1	4	43 (27)
2010	274,224	277,855	33,888	1.20	26.1	28	6	7	41 (25)

[a] Inst., Instrument; PI, Principle investigator; CHE, Chemistry Division; DMR, Division of Material Research; MCB, Molecular and Cellular Biosciences Division; *N*, number, USD, U.S. Dollars.

Table 2. Comparison of Awards with Equipment Support for New and Experienced PIs[a]

Fiscal Year	Awards to New PIs, %	Average Award to New PIs, USD	Average Award to Exp PIs, USD	Average Inst Award to New PIs, USD	Average Inst Award to Exp PIs, USD	Budget for Inst for New PIs, %	Budget for Inst for Exp PIs, %	New PIs with Inst, %	Exp PIs with Inst, %
2005	50	265,448	307,858	27,480	45,589	10	15	83	69
2006	48	242,350	289,352	19,703	26,367	7	9	67	46
2007	59	247,646	321,755	33,137	18,954	13	6	55	64
2008	51	289,562	307,840	48,384	35,090	17	11	63	58
2009	60	231,035	288,147	23,611	23,920	12	8	69	53
2010	56	251,369	321,183	38,473	24,144	15	8	73	44

[a] Inst., Instrument; PI, Principle Investigator; Exp, experienced, USD, U.S. Dollars.

Table 3. Distribution of Instrument Types in RUI Awards to Faculty in Chemistry and Biochemistry Departments[a]

Instrument	FY 2005	FY 2006	FY 2007	FY 2008	FY 2009	FY 2010	Total
Spectrophotometer	4	5	2	7	3	3	24
Microwave Reactor	1	1	2	0	2	1	7
Computational Hardware	4	2	1	3	4	4	18
Chromatography	4	1	5	3	2	5	20
Laser System	2	1	1	2	1	1	8
Photolysis System	1	1	1	1	1	0	5
Microscope/Centrifuge	1	1	2	0	2	1	7
DSC/TGA	0	0	1	1	3	0	5
X-ray System	0	0	0	0	1	1	2
General	2	2	5	5	8	9	30
Total Number per Year	19	14	20	22	27	25	127

[a] FY, Fiscal Year; DSC, Differential Scanning Calorimeter; TGA, Thermal Gravimetric Analysis.

increasing from around 25 awards in 2005−2006 to about 40 awards in recent years. The average award amounts to $267,965 and has an initial duration of typically two or three years. However, most award applicants requested at least a one-year unfunded extension so that the average duration of awards made in 2005−2007 is about four years. Awards made after 2008 are still active and have not needed to request the extra one or two years of unfunded extension. Most were single investigator awards, although about one out of every three or four awards had a co-PI. The column on the far right of Table 1 reports the total number of awards each year; the number in parentheses is the number of awards requesting equipment.

Table 2 shows data for awards that had requested an instrument. The percentage of all awards to new PIs each year is just over 50% and, as expected, the average dollar award to new PIs, $252,389, is about $15,000 less than the average of all awards and about $54,000 less than the $306,023 average award with an instrument to experienced PIs. However, the average dollar amount requested for equipment is somewhat larger for new PIs, especially in the 2007−2010 time frame. This may reflect the realization that new PIs need equipment to set up and initiate a research program. Consistent with this trend is the larger percentage of the total budget allocated to equipment for new PIs compared to experienced PIs. The last two columns in Table 2 illustrate that, in general, a larger percentage of new PIs requested equipment compared to experienced PIs.

Many faculty are interested in funding rates for proposals submitted to the Chemistry Division at NSF. During the NSF Town Hall meeting at the Spring 2011 ACS National Meeting, the funding rates for all research proposals submitted to the Chemistry Division for fiscal year 2010 were summarized as follows: For new PIs or for experienced PIs who were not funded

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from the previous two submissions, the funding rate is 17%. For PIs who were previously funded but subsequently received one declination, the success rate is 44%. Renewals have a 54% success rate and are proposals from PIs who have been supported by the Chemistry Division and are requesting subsequent support. In 2010, the Chemistry Division received 1644 research proposals and the overall funding rate was 24%. RUI proposals, regardless of whether or not an instrument was requested, have success rates similar to the overall success rates within the Chemistry Division at NSF.

■ HISTORICAL OVERVIEW 2005−2010 OF THE INSTRUMENTS FUNDED BY THE RUI PROGRAM FOR CHEMISTRY AND BIOCHEMISTRY FACULTY

Table 3 summarizes the types of instruments requested in RUI awards for the period 2005−2010. Spectrophotometers include UV−visible, IR, microwave, fluorometers, and so on: this is the most frequently requested instrument category. The next most frequently requested category of instruments was chromatography, including gas-, liquid-, and ion-chromatographs. Nearly 15% of all award applications requesting support for an instrument also requested computer hardware (workstations or clusters), illustrating the growing importance of computational chemistry. It is likely that these computational resources will be heavily used in traditional teaching courses. The "general" category includes rotary evaporators, incubators, constant temperature shakers, hydrogenation systems, vacuum gloveboxes, solvent purification systems, and so on.

■ CONCLUSION

Nearly two-thirds of all RUI awards support equipment acquisition associated with the research project. At many undergraduate institutions, these instruments will also be used for some specialized laboratory experiments and the computer workstations or clusters will likely enjoy significant use by students in traditional classroom settings. As with the MRI program, PIs submitting to RUI must have a strong track record of productivity evidenced by publications in research journals. PUI chemistry faculty considering submitting an RUI proposal are encouraged to visit the Web site[5] for the CHE, DMR, or MCB division to learn about funded RUI proposals, or to call the appropriate NSF program officer with specific questions about the proposed research.

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JOURNAL OF CHEMICAL EDUCATION

REPORT

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Role of Undergraduate Research in an Excellent and Rigorous Undergraduate Chemistry Curriculum

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ABSTRACT: In 2008, the American Chemical Society's (ACS) Committee on Professional Training (CPT) issued new guidelines for the approval of undergraduate chemistry programs. Undergraduate research continues to meet the requirements for a portion of laboratory hours necessary for a certified degree, and can also contribute to the in-depth course requirements. The CPT supplements on undergraduate research, excellent and rigorous undergraduate programs, and student skills expand on the pedagogical advantages of undergraduate research. The ACS Guidelines and CPT supplements are supportive of the important role that a research-supportive curriculum can play in the development and training of chemistry students. Departments interested in establishing or strengthening research-rich environments can find support from the ACS Guidelines and from the resources available through the Council on Undergraduate Research (CUR).

KEYWORDS: Curriculum, Undergraduate Research

FEATURE: Association Reports

The American Chemical Society's (ACS) Committee on Professional Training (CPT) published new guidelines in 2008 for the approval of undergraduate chemistry programs.[1] The attributes of "excellent"[2] and "rigorous"[3] undergraduate programs are further detailed in two supplements on this topic. Several aspects of the ACS Guidelines facilitate undergraduate participation in research. The guidelines identify curricular requirements, including foundational and in-depth courses leading to an ACS-certified degree. Undergraduate research can be used to meet one of the in-depth courses in a semester system (up to four credit hours) and a commensurate number of credit hours in a quarter system. Students must complete 400 laboratory hours for the certified degree. Up to 180 hours of research participation can be used to fulfill this requirement, with completion of a comprehensive written research report. Of particular interest to faculty who wish to promote undergraduate participation in research is that policies and statements in the new guidelines embrace the value of undergraduate research in a scope and form completely consonant with the longstanding mission and views of the Council on Undergraduate Research (CUR).

FEATURES OF UNDERGRADUATE RESEARCH

CUR has adopted the following definition of undergraduate research:[4]

> An inquiry or investigation conducted by an undergraduate student that makes an original intellectual or creative contribution to the discipline.

Two important points in this definition are that undergraduates should conduct original work and that the outcomes of the research, if successful, represent a contribution to the discipline. Undergraduates should not merely repeat work that has been done before, but need to be involved in an exploration of something new. Implicit in the expectation that the research represents a contribution to the discipline is that the outcomes be disseminated within the discipline by the usual means. While a common means of research dissemination involves conference presentations, the ultimate means of dissemination in chemistry is through peer-reviewed publications in disciplinary journals. The ACS Guidelines mandate that the student write a comprehensive report on her or his research project if the research is used as an in-depth course or toward the 400 laboratory hours. The ACS Guidelines and a supplement on undergraduate research[5] specify that research projects represent original work, develop new knowledge, and are envisioned and undertaken with the intent to contribute toward a peer-reviewed publication.

CPT'S RIGOROUS AND EXCELLENT PRACTICES

To provide additional guidance with regard to effective educational practices, the CPT has described the characteristics of excellent and rigorous undergraduate chemistry programs that are supportive of many aspects of the undergraduate research enterprise. An important aspect of program excellence relates to the development of professional skills in students, which is also an expectation for approved program that is new in the 2008 Guidelines. The curriculum of an excellent program develops in students the ability to communicate in oral and written form, work in teams, ask questions, design experiments, interpret results, think in innovative ways, exhibit leadership, develop a desire for lifelong learning, and behave in an ethical manner.

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Related to excellent educational pedagogy, a curriculum should include integrative curricular experiences in which students apply knowledge in new contexts and transfer knowledge from one context to another. Finally, among the characteristics of an excellent faculty is a willingness to interact with students in the learning process. Faculty will recognize that the CPT's meaning of excellence is fully incorporated into a high-quality undergraduate research experience.

Regarding the meaning of program rigor, the CPT supplement speaks of the desirability of experiences that allow students to apply fundamental principles toward an understanding of chemical systems. Learning environments that actively engage students, promote a progressive development in the student's own responsibility for learning, and promote the development of critical thought and multistep problem solving are desired. The document speaks of the need for students to be able to analyze data and scientific arguments, synthesize and apply concepts from multiple subdisciplines of chemistry, and recognize the applicability of foundational and advanced concepts to new situations. The value of communication skills in both oral and written form is emphasized in a rigorous curriculum. Those familiar with CUR and its mission would argue that a high-quality undergraduate research experience embodies the characteristics that CPT ascribes to a rigorous program.

CPT recognizes the pedagogical value of undergraduate research and it appears as a thread throughout the ACS Guidelines and the committee's supplements. The guidelines point out that undergraduate research allows students to integrate learning experiences and participate directly in the process of science. The supplement on the characteristics of a rigorous program indicates that undergraduate research has the potential to facilitate in students a mastery of independent thought and self-direction.[3] The supplement on undergraduate research[5] recognizes that research can often be the most educationally valuable experience for students and that students participating in research have the potential to grow professionally and personally in a manner that is not possible through traditional classroom and instructional laboratory experiences. A supplemental document on student skills describes undergraduate research as one of the most powerful opportunities for students to learn problem-solving skills and provides a unique opportunity for students to develop oral and written communication skills.[6]

However, a research experience should not be viewed as the primary place where these skills and competencies are developed. Instead, it may be more effective to have a curriculum that includes activities that allow students to progressively develop these skills. What would such a curriculum look like? Students should learn to work in teams and have opportunities to develop leadership skills, which means that teamwork should be a part of classroom and laboratory activities. Written and oral communication skills should be developed throughout the curriculum, with increasingly advanced expectations of the students as to the quality and scope of various forms of communication. In addition to writing, students should be able to read and properly use the primary chemical literature; a variety of approaches can be integrated into the curriculum to develop these skills. Approaches for instruction in professional ethics can include a guest lecture program, a separate course, or integration of ethics broadly across the curriculum.[7] Instructional laboratory experiences that allow students to develop testable hypotheses, design experiments, ask questions, interpret data and draw and argue for certain conclusions should occur at both the foundational and in-depth level. For examples of such activities, CUR has published a compendium on developing and sustaining a research-supportive curriculum.[8]

Stressed throughout both the CPT supplements on rigorous and excellent undergraduate programs is an emphasis on assessment to ensure that students are learning and developing in the ways the faculty have intended. While CUR and others have been advocates for the educational value of having undergraduates participate in original research, for many years no thorough assessment studies substantiated these claims. Recently, assessment data from carefully constructed studies have become available that support the benefits of student participation in research.[9,10] The Student Assessment of their Learning Gains (SALG),[11] Survey of Undergraduate Research Experiences (SURE),[12] and Classroom Undergraduate Research Experience (CURE)[13] are assessment instruments that can be used to evaluate the effectiveness of research and research-like experiences. From CPT's perspective, the research reports that ACS-approved departments provide with their periodic report materials are an important form of assessment. Because these usually describe the result of a capstone project completed toward the end of the students' undergraduate studies, the extent to which the reports are thorough, well-referenced, and indicate that the students have devoted a considerable amount of time and thought to the project helps the committee evaluate the degree to which excellence and rigor are emphasized in the research experience and the program.

■ ACS GUIDELINES SUPPORT UNDERGRADUATE RESEARCH

In a mutually reinforcing way, both CUR and the CPT documents can be helpful to departments and institutions seeking to enhance research in their curriculum. For smaller departments at predominantly undergraduate institutions, the ACS Guidelines have served as an important source of leverage over the years for enhancing the institutional support for the department. Many aspects of the ACS Guidelines relate to infrastructure and institutional support that help support excellence and rigor in undergraduate research programs. While the ACS Guidelines do not mandate any particular number of support staff, they do speak to the importance of support staff for stockroom administration and equipment maintenance as a way of allowing faculty members to devote their time and effort to academic and scholarly activities. The guidelines place a maximum of 15 contact hours in the classroom or laboratory or in combination for any faculty member, but also state that the actual number should be significantly smaller, particularly for faculty members who supervise undergraduate research. The guidelines also speak to the importance of the quality of the physical plant and instrument holdings. For example, ACS-approved programs must have a functioning NMR spectrometer as well as a range of other equipment. Finally, the guidelines emphasize the importance of professional development activities, including the need for professional development opportunities for faculty and staff, such as travel support for attendance at professional meetings and opportunities for sabbatical leaves. CUR has also been an advocate for providing the support and infrastructure that is needed to initiate and sustain a research program that involves undergraduates. CUR offers numerous publications including the *CUR Quarterly* journal as well as workshops to help individuals and institutions develop excellent

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undergraduate research programs. Readers are encouraged to visit the CUR Web site for more information: http://www.cur.org/ (accessed Nov 2011).

The 2008 ACS Guidelines and supplement documents encourage the participation of undergraduates in research and make it clear that the goals of an excellent and rigorous undergraduate chemistry programs are embodied by the same features that characterize a high-quality undergraduate research experience.

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JOURNAL OF CHEMICAL EDUCATION

REPORT

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Highlights from *The Science Teacher* Welcomes Two New Contributors

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ABSTRACT: This article provides a brief history of the *Journal of Chemical Education*'s Highlights from *The Science Teacher* feature column, bids farewell to its long-time contributor Steve Long, and introduces two new contributors to the column.

KEYWORDS: High School/Introductory Chemistry, Professional Development

FEATURE: Reports from Other Journals

CHANGES AHEAD FOR HIGHLIGHTS FROM *THE SCIENCE TEACHER*

For over a decade, the *Journal of Chemical Education* (*JCE*) feature, Highlights from *The Science Teacher*, has provided readers with synopses of recent *The Science Teacher* (*TST*) articles of interest to chemical educators and has connected these articles to additional related resources in *JCE*. *TST* is a peer-reviewed journal published by the National Science Teachers Association (NSTA) for its high school teacher members. As *TST* includes material from all areas of science, not only chemistry, the *JCE* feature provides an easy way to view highlights of *TST* articles most relevant to chemistry teachers.

Steve Long, a high school chemistry teacher, has been at the helm of this feature since its introduction in the *Journal* through his last column in 2010 (Box 1). A call for new contributors to the column[1] resulted in the selection of both Carole Magnusson and Susan Reslewic to carry on the Highlights column. This article shares Steve's parting words and introduces Susan and Carole to readers.

Steve Long Bids Farewell

After 12 years (beginning in June of 1998) and 21 articles synopsizing chemistry articles in *The Science Teacher* (*TST*) for *JCE* readers, it is time for me to pass the responsibility for this effort to new writers. Emory Howell, *JCE* High School Editor at that time, knew me from training together in the Operation Chemistry program. Aware of my involvement with the NSTA, he asked me to begin writing a new *Journal* feature highlighting relevant articles in *TST*. Little did I realize how much I would grow and learn in both content and pedagogy as I read, researched, and referenced hundreds of articles in *TST* and *JCE* through these years. I've worked with a great and supportive staff at *JCE*, including Howell, Betty Moore, and Erica Jacobsen. However, I think it is time for a fresh approach and new ideas. Originally, this was part of an effort to strengthen the *Journal* offerings for high school readers (although I hope that others have found it useful as well). The challenge for *JCE* continues to be that of finding effective methods for sharing its wealth of resources and information with current high school teachers and embracing even more readers. With the superb *JCE* staff and talented new writers for this project, accomplishing this challenge should be manageable. Thank you for the opportunity to have served you through my articles.

Carole Magnusson Introduces Herself

I have not always been a teacher: I started teaching only 25 years ago. After graduating from the University of California at San Diego with a B.A. in chemistry, I worked for seven years at Scripps Institute of Oceanography in San Diego as a lab technician, analyzing shell and water samples for the isotopic ratios C^{13}/C^{12} and O^{18}/O^{16}. In my late twenties, I went back to school and earned a teaching credential in the physical sciences, which allowed me to teach chemistry, physics, and physical science at the high school level.

During my first several years of teaching, I taught all three subjects; for the past several years, however, I've taught only chemistry. In the early 1990s, I became acquainted with the Chemistry in the Community (ChemCom) curriculum, which I have found quite effective for teaching chemistry. In fact, I currently teach five classes of ChemCom, and hope to teach it for the rest of my career.

I began my teaching career at Sacramento High School in Sacramento, California. When Sacramento High School converted to a charter school in 2003, I moved to the brand new high school in Sacramento, Rosemont High School. I had the unique opportunity to open a new school and start a new science department as the department chair.

Although teaching does take up a large part of my day, I do enjoy being involved in activities outside of school. I look forward to working on this project.

Susan Reslewic Introduces Herself

I am delighted to join Carole Magnusson and the *Journal of Chemical Education* as a reviewer of articles in *The Science Teacher*

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1. Highlights from *The Science Teacher*. December 2009 to Summer 2010. **2010,** *87* (12), 1286–1289; DOI: 10.1021/ed100890u.
2. *The Science Teacher*. March 2009 through November 2009. **2010,** *87* (3), 239–242; DOI: 10.1021/ed800103k.
3. *The Science Teacher*. Spring 2008. **2008,** *85* (6), 772–774; DOI: 10.1021/ed085p772.
4. *The Science Teacher*. Fall 2007. **2007,** *84* (11), 1748–1749; DOI: 10.1021/ed084p1748.
5. *The Science Teacher*. Spring 2007. **2007,** *84* (4), 582–583; DOI: 10.1021/ed084p582.
6. *The Science Teacher*. Spring 2006. **2006,** *83* (6), 830–832; DOI: 10.1021/ed083p830.
7. *The Science Teacher*. Fall 2005. **2005,** *82* (11), 1609; DOI: 10.1021/ed082p1609.
8. *The Science Teacher*. Spring 2005. **2005,** *82* (4), 514–515; DOI: 10.1021/ed082p514.
9. *The Science Teacher*. Fall 2004. **2004,** *81* (9), 1254–1255; DOI: 10.1021/ed081p1254.
10. *The Science Teacher*. Spring 2004. **2004,** *81* (4), 462–463; DOI: 10.1021/ed081p462.
11. *The Science Teacher*. Summer 2003. **2003,** *80* (9), 987; DOI: 10.1021/ed080p987.
12. *The Science Teacher*. Winter 2003. **2003,** *80* (2), 135–136; DOI: 10.1021/ed080p135.
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Box 1. Contributions by Steve Long in the *Journal of Chemical Education* synopsizing articles from *The Science Teacher* relevant to high school chemistry teachers, 1998–2010.

of interest to *JCE* readers. Most recently, I've taught high school biology and chemistry at the Ramaz School in New York City, and I direct the design and production of chemistry content for the company Virtual Nerd, a provider of online video-based tutorials in math and science. My interests center on integrating new communication and visualization technologies into science, and especially chemical, education. I am an avid reader of *JCE,* and often apply insights and ideas from material in *JCE* to my work in the classroom.

A background in science rich with opportunities to appreciate and communicate diverse applications of chemistry has nurtured my passion for chemical education. After falling in love with chemistry as a sophomore in high school in Newburyport, Massachusetts, I majored in the subject at Princeton University (1999), choosing to perform chemical analyses on a set of artifacts from pre-Columbian Peru for my senior thesis project. Fascinated by the power of analytical chemistry to tell stories about materials and contexts seemingly unrelated to the discipline, I continued research at the University of Wisconsin—Madison, receiving a Master's degree in Anthropology under the direction of the Laboratory for Archaeological Chemistry, and a Ph.D. in Chemistry (2005) for research that identified patterns in the human genome using fluorescence microscopy.

After graduate school, preference for the communication- and application-based parts of science led me to join the pharmaceutical arm of the consulting firm McKinsey and Company. In 2008, I left consulting to pursue an entrepreneurial opportunity in science education and video production. I began teaching at the Ramaz School in 2009, and continue to find chemical education full of thrilling chances to communicate and excite others about science. I am particularly excited about the opportunity to use widespread and ever faster communication technologies to broaden access to science education.

In Fall 2011, I joined the faculty of Manhattan College as a Visiting Professor of Chemistry. I look forward to helping communicate advances in science education by contributing to the *Journal of Chemical Education.*

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JOURNAL OF CHEMICAL EDUCATION

NEWS AND ANNOUNCEMENTS

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News and Announcements Changes: Share Your News Online with the Chemical Education Community

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ABSTRACT: Changes in the dissemination of News and Announcements at the *Journal of Chemical Education* are described. Most saliently, these include instructions for contributing news or an announcement relevant to the chemical education community through the online form at the American Chemical Society Division of Chemical Education Web site.

KEYWORDS: General Public, Communication/Writing, Administrative Issues

As part of ongoing changes at the *Journal of Chemical Education* (*JCE*), the function and publication of News and Announcements in the pages of *JCE* is transforming. The partnership between *JCE* and the Division of Chemical Education (DivCHED, a technical division of the American Chemical Society—ACS) has evolved to the point that changes can (and should be) made in the way news and announcements are handled in the *Journal*. DivCHED already provides timely information to the chemical education community via the DivCHED Web site,[1] the *CHED Newsletter*,[2] and the DivCHED Facebook open group.[3,4] Anyone with news or an announcement relevant to the chemical education community can contact DivCHED through the online form at http://www.divched.org/content/contribute-website and, following approval, information will be disseminated online.

Beginning with Volume 89, the *Journal* will use the News and Announcements designation to publish occasional items, mainly to communicate with our readers about changes underway at *JCE*. Watch for these, and connect with the news of your community online via DivCHED!

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JOURNAL OF CHEMICAL EDUCATION

BOOK AND MEDIA REVIEW

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Review of *Principles of Chemistry: A Molecular Approach*

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Principles of Chemistry: A Molecular Approach by Nivaldo J. Tro. Prentice Hall: Upper Saddle River, New Jersey, 2010. xviii + 870 pp. ISBN: 978-0321560049 (paper). $170.

The market for chemistry textbooks seems both crowded and fractured. Numerous texts abound for "preparatory" classes, "GOB" classes, "liberal arts" classes, one-semester "introductory" classes, two-semester classes for chemistry majors, two-semester classes for science majors, advanced texts for "honors" classes, and perhaps some others of which I am not aware. Nivaldo Tro's *Principles of Chemistry* is suitable for any two-semester class.

Books for two-semester general chemistry courses typically come in two lengths: a long version, often with only the word "chemistry" in its title, and a shorter version, usually with "principles of" added. Such is the case with the textbook under review here. Tro's *Principles* is four chapters shorter than its parent text:[1] Biochemistry; Chemistry of the Nonmetals; Metals and Metallurgy; and Transition Metals and Coordination Compounds have been omitted. In addition, small sections of other chapters have also been sacrificed, as well as special topic boxes and end-of-chapter review questions. The exercises at the end of the chapter appear to be the same. Comparing the chapter on gases from the long and short version will illustrate the differences: the *Principles* version lacks short sections on the description of a manometer, deep-sea diving and partial pressures, kinetic molecular theory and the ideal gas law (which mathematically derives the ideal gas law from postulates of kinetic molecular theory), and chemistry in the atmosphere (covering air pollution and ozone depletion). Side boxes on "chemistry in medicine" dealing with blood pressure and "chemistry in your day" on snorkeling are also removed.

The book's organization is similar to other textbooks, but it is not an "atoms first" text. Balanced chemical equations, stoichiometry, and thermochemistry are all covered before the quantum mechanical details of atoms and bonding. Also, the chapter on gases comes earlier than in many books, falling between the chapter on stoichiometry (Chapter 4) and thermochemistry (Chapter 6), rather than being placed just before liquids and solids (Chapter 11). Whatever your preference, the chapter on gases can be covered in either location without losing clarity, or if desired, the book can be customized through the publisher. Organic chemistry and biochemistry are mildly integrated throughout the text. For example, organic compounds are distinguished from inorganic compounds and examples of a few hydrocarbons are given in Chapter 3, which deals with nomenclature. However, naming rules for basic hydrocarbons and functional groups are segregated in the book's final chapter. I do not have a problem with this, but those who prefer fuller integration should take note.

In general, the writing is clear and concise, and the presentation of topics within a chapter usually follows a logical progression. I thought the chapter on the quantum-mechanical model of the atom (Chapter 7) was particularly good; several students commented favorably on the quantum-mechanical strike zone analogy. Each chapter begins with a short section that introduces a real-life application of the chemistry covered in the chapter. For example, the chapter on stoichiometry begins with a discussion of fossil fuel combustion and global warming. I am not sure students are always prepared to understand the chemistry introduced in these sections, but the idea is to capture interest, not teach content. From this introduction, the content of the topic unfolds in a logical and straightforward way. Worked sample problems are included with almost every section and questions testing conceptual understanding are sprinkled throughout each chapter. All chapters conclude with a list of key terms, a summary of key concepts, a list of important equations or relationships, and a list of skills students should acquire.

Other design features are deserving of comment: Graphics connecting macroscale and nanoscale are common. Sample problems are solved in paired columns with the verbal explanation of what's being done in one column and the numerical manipulation in the other. A similar style is used to illustrate algorithmic procedures (e.g., drawing Lewis structures or solving equilibrium problems). Lastly, the book can be purchased with access to the publisher's online course management program, MasteringChemistry, reviewed previously in this *Journal*.[2]

In the end, Tro's text is a worthwhile addition to the current crop of textbooks for the two-semester market.[3] Some may not like the stripped down style, in which case the extended version might be more suitable. Personally, I am not sure that students pay much attention to the boxes unless their teachers do, in which case the boxes seem superfluous; sometimes all the additions can confuse the narrative. Instructors who prefer a more data-driven text or one with more integrated connections to current issues will already know the texts they favor; Tro's book is in another category.

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(3) Editor's Note: Publication of the second edition of *Principles of Chemistry: A Molecular Approach* is expected January 2012.

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JOURNAL OF CHEMICAL EDUCATION

BOOK AND MEDIA REVIEW

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Review of *Food Chemistry*, 4th Revised and Extended Edition

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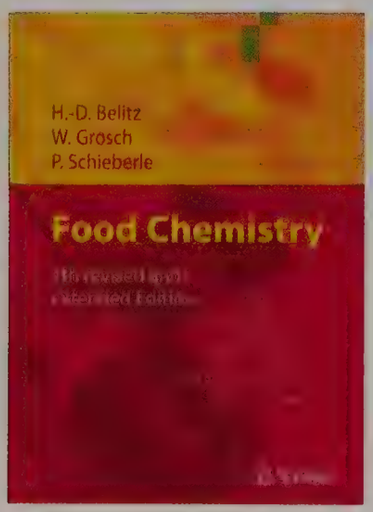
Food Chemistry 4th revised and extended Edition by H.-D. Belitz, W. Grosch, and P. Schieberle. Springer Verlag: Berlin, Germany, 2009. 1070 pp. ISBN: 978-3540699330 (cloth). $239.

This is the fourth edition of *Food Chemistry* to be published in English and is the translation of the sixth edition published in German. The first edition was written in 1982 to fill a need for a comprehensive text on the chemistry of foods. Each of the 24 chapters covers different components of food from macronutrients including proteins, carbohydrates, and lipids, to minor constituents including vitamins, minerals, flavors, and food additives. After discussing the main constituents of foods in the early chapters, the remainder of the book covers categories of foods. The table of contents lists not only the chapter titles, but also all of the subtitles within a chapter, providing a good overview of the book and the flow of topics within a chapter. The book is also well indexed, providing a quick way to find specific information.



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It is interesting to note the first and last chapters are about water. The first chapter discusses water as a molecule. In many foods, it is the single largest component, making up from 65−90% of fruits, vegetables, meats, and milk. Water is involved in many of the chemical reactions in foods and being able to control it is often an important way to preserve or extend the shelflife of foods. The final chapter is on water as a beverage that we drink and discusses the different types of water available.

Information on food processing and nutrition is provided as it directly relates to the chemistry of the foods and can aid in an understanding of food products. In many cases, chemical reactions caused during food processing can alter the state of the food, changing the texture, color, or flavor of the product. The book is not intended to be a reference for food processing techniques or nutrition, although it does well at incorporating useful information on these topics where appropriate.

Food Chemistry includes a number of topics that have been discovered or become important over the past few years. As in any field, our knowledge continues to grow and expand as we continue to do research. Allergens have always been present in food; however, the incidence of food allergies has grown dramatically and is an important consideration in processed foods. Acrylamide formation in foods was discovered within the past 10 years and has been the subject of extensive research. Other topics that have gained importance in understanding food chemistry are phytosterols and glycemic index. These are just some of the completely new areas that the book covers.

There are other topics that were included in previous editions that have been expanded, owing to increased knowledge and increased importance. As our analytical techniques have become more sophisticated, we have learned more about food contaminants that we previously were not able to detect. Other topics that the book addresses in more detail are phenolic compounds, alcoholic beverages, and tea and coffee. Phenolic compounds are present in plant foods and their antioxidant activity makes them important from a nutritional standpoint as well as physiologically. This is a particularly active area of current research to JE_je-2011-00597hunderstand if and how they may be involved in disease prevention. As the evidence grows, it will be important to understand how phenolic compounds react and how they can be protected in foods.

Food Chemistry does an excellent job of explaining the chemistry of food constituents as well as finished food products. As a textbook, it provides the basis for an in-depth course on food chemistry. The book was written by food chemistry professors and is organized in the same way they teach their course. A course based on the book would not be limited to those pursuing the field of food science. Any person who needed a chemistry course as part a science requirement in another field could benefit. All people can relate to food and it provides an excellent medium to present basic chemical principles. The wealth of knowledge in the book makes it a good reference book for anyone working in the field of food science.

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BOOK AND MEDIA REVIEW

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Review of *Kinetic Modeling of Reactions in Foods*

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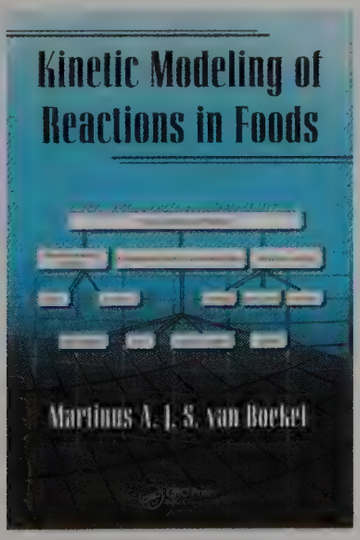
Kinetic Modeling of Reactions in Foods by Martinus A. J. S. van Boekel. CRC Press: Boca Raton, Florida, 2009. 767 pp. ISBN: 978-1574446142 (cloth). $195.

Kinetic Modeling of Reactions in Foods is a welcome addition to the food chemistry and food science literature. The author's stated aim is "to introduce appropriate kinetic models and modeling techniques that can be applied to food science and technology"; this book is an admirable contribution toward that end.

Throughout the book, the focus hones in on how to capture changes in food quality attributes within mathematical models. These attributes are typically based on quantifiable physical, biochemical, and microbial indicators. In this sense, the author deals with the breadth of kinetic modeling, rather than limiting the coverage to a single discipline (e.g., chemical kinetics). van Boekel does a nice job of combining this into a single text—albeit a long one (~750 pages). Each of the presented topics is provided in reasonable detail starting with background coverage and ending with a thorough and well-organized Bibliography and Suggested Further Reading section.



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The text is divided into two sections: (i) The Basics; and (ii) Application of the Basics to Chemical, Biochemical, Physical, and Microbial Changes in the Food Matrix. Chapters within the first section provide a solid introduction to the concepts of modeling and kinetics. Early on, the author presents some of the vocabulary of modeling, explaining concepts such as empirical versus mechanistic modeling and pointing out how these two approaches represent extremes that are blended when modeling food systems. Kinetic models are generally introduced from a mechanistic perspective, including rationales based on temperature, pressure, and charge effects on chemical reactions. An introductory chapter on thermodynamics is included as background material for subsequent chapters. Concluding the first section of the book is a lengthy chapter dealing with the role of statistics in kinetic modeling. The author argues that modeling is an iterative exercise for which statistics can provide valuable information as one proceeds through iterative cycles.

The applications section of the book deals with the breadth of issues one would expect when considering food quality. This includes chemical and enzyme-catalyzed reactions, microbial growth and inactivation, and changes in physical properties. The author clearly explains the differences that one may encounter when working with "clean" experimental systems versus actual foods. For example, there are observed differences in protein denaturation kinetics when comparing purified proteins in model buffer systems and the same proteins in a food matrix. An important chapter in this section—one that is unique relative to many modeling texts—deals with the complexities of working with food matrixes. Most of the models presented throughout the text are attempts to apply fundamental chemical principles to complex food systems. This chapter directly addresses the issue of translating results from model studies to those expected for real foods.

The book emphasizes mathematical modeling rather than conceptual modeling and, thus, requires some mathematical competency. My impression is that such competency could be attained from introductory courses in calculus, matrix algebra, and statistics. The author succeeds in verbalizing the conceptual basis of the presented equations so readers are guided through many of the mathematical arguments.

I recommend this book for those interested in applying mathematical modeling techniques to foods and other systems with comparable matrixes. This includes (advanced) university students, established researchers, and those doing industrial modeling. The subject matter is complex so one should not expect a "light read"; at least that is not my experience. However, the author presents the material in a thoughtful and engaging manner. Importantly, the book is quite well referenced, making it relatively easy to find supplemental material when desired.

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JOURNAL OF CHEMICAL EDUCATION

ARTICLE

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The 2010 Rankings of Chemical Education and Science Education Journals by Faculty Engaged in Chemical Education Research

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ABSTRACT: Faculty active in chemical education research from around the world ranked 22 journals publishing research in chemical education and science education. The results of this survey can be used to supplement impact factors that are often used to compare the quality of journals in a field. Knowing which journals those in the field rank as top tier is advantageous in academic environments that require researchers to publish often and for greatest impact.

KEYWORDS: Graduate Education/Research, Chemical Education Research, Professional Development

FEATURE: Chemical Education Research

INTRODUCTION

The current research environment in academia is one that demands productivity. Faculty members are expected to publish, with many universities showing an increase in the rate of publication over the past 30 years.[1] In Australia, for example, universities receive extra funding based on their academic publication rates, and promotion can be difficult with a low publication record.[2] In the United States, publication records are used to gauge scholarly output and are a metric often used in making promotion and tenure decisions.[3]

Pienta has documented publication rates for chemistry education active faculty and has compared them to all faculty within their departments.[4] The average publication rate per year of chemistry education articles for faculty active in chemistry education is 0.67 for those at doctoral degree-granting institutions and 0.55 for masters degree-granting institutions. The publication rates for all faculty members within the same departments as faculty active in chemistry education are 3.7 and 1.2 for doctoral degree-granting and masters degree-granting institutions, respectively. The low annual publication rate in chemistry education as compared to faculty colleagues publishing in chemistry and science research journals can be attributed to the time required to conduct research and prepare manuscripts in chemistry education. Timely review and publication is critical to the success of researchers and knowledge about the citation metrics and standing of a journal as determined by those in the field becomes vital. The prevailing viewpoint among scientists is that articles appearing in higher-quality journals tend to have greater impact.[5]

In most science disciplines, including chemistry, the impact factor has become a widely used metric to distinguish among journals in terms of influence and prestige. Eugene Garfield designed the impact factor in 1963 and founded the Institute for Scientific Information (ISI) to tabulate impact factors.[3,6] ISI is now part of the publishing company Thomson Reuters, which calculates impact factors annually for the journals it indexes and publishes these factors in Journal Citation Reports (JCR).[3,6]

Calculating Journal Impact Factor

The impact factor of a particular journal is the frequency an average article is cited in a specific time period. Frequently it is determined for a two-year time frame, but can be calculated for a five-year period as well. To calculate a two-year impact factor of a specific indexed journal, the number of articles published over a two-year period is counted. The number of citations of those articles in the following year is counted. The impact factor is the ratio of citations to the number of articles published in that two-year period.[6] Thus, a two-year impact factor of 5.0 would mean that the average article in a specific journal is cited five times in two years.

In computing the total number of articles published, JCR includes only articles, reviews, and notes, while the total number of citations can also include letters and meeting abstracts.[3] Although JCR publishes a two-year impact factor, in some fields a five-year impact factor may be a more appropriate metric. Fields that have lengthy submission-to-publication schedules, and take longer than two years to integrate and respond to publications may be better served by an impact factor which sums citations and the numbers of articles published over a longer time frame.

Thomson Reuters calculates impact factors for over 10,000 journals from 60 countries; a vast majority of those journals are published in English or at a minimum the bibliographic information is in English.[6] However, impact factors are not available for all journals in a particular field. For example, in the field of chemistry education, *The Chemical Educator* and the *Australian Journal of Education in Chemistry* are not indexed by the ISI.[6] In science education, the *Journal of College Science Teaching* is not indexed by the ISI.[6] Impact factors, while based on a mathematical formula, are not without bias. Heavily cited review articles can inflate the impact factor of a journal, though review articles can be removed from the calculation. Impact factors can also disfavor research fields that are small in size or fields that tend to

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Table 1. 2009 Two-Year and Five-Year Impact Factors with Immediacy Indices for Chemistry Education and Science Education Journals[a]

Journal Title	Two-Year Impact Factor	Five-Year Impact Factor	Immediacy Index
Chemical Education Journals			
Chemistry Education Research and Practice	0.742	None[b]	0.345
Journal of Chemical Education	0.586	0.677	0.226
Biochemistry and Molecular Biology	0.292	0.474	0.174
Science Education Journals			
Journal of Research in Science Teaching	1.910	2.805	0.434
Science Education	1.625	2.800	0.489
Research in Science Education	1.088	1.313	0.861
International Journal of Science Education	1.047	1.614	0.096

[a] See ref 6. [b] The five-year value was not listed for *CERP* within JCR.[6]

reference older studies or nonjournal sources, such as the humanities or the social sciences.[7,8]

Despite the shortcomings of impact factors, they are used by academic institutions to compare journals within a field and to evaluate a scholar's work.[6,10] Comparison of impact factors across journals and fields can be misleading, as a low impact factor would be impressive in a small, specialized field, but not in a large, general field.[6] There has also been little published regarding the validity of impact factors as indicators of a scholar's work.[3,9,11]

■ IMPACT FACTORS FOR JOURNALS PUBLISHING CHEMISTRY EDUCATION RESEARCH

The 2009 impact factors for indexed journals that publish chemistry education research in chemistry education and in science education are listed in Table 1.[6]

By way of comparison, Table 2 lists impact factors for journals that publish a broad range of science research and chemistry research, including those that focus on specific science disciplines.

The two-year and five-year impact factors for journals in chemistry including *Science* and *Nature* are substantially higher than chemistry education and science education research journals. Faculty in chemistry who evaluate the scholarship of chemistry education researchers through promotion and tenure committees, salary and merit committees, and so on, need to have a method by which they can fathom the low impact factors in the field of chemistry education.

Every two-year and five-year impact factor for chemistry education specific journals is less than 1.000. Thus, the values are nearly meaningless as a method of comparing the prestige of publishing in a given journal. The immediacy index is defined as the number of citations to articles in a given year divided by the number of articles published in that journal. Thus, it represents the average number of times an article is cited in the year that it is published. The low immediacy indices values in Table 1 indicate that integration of published work in the field of chemistry education research is longer than one year because in every case the immediacy index is less than one.

Table 2. 2009 Impact Factors for *Science*, *Nature*, and Selected Journals in Chemistry Subdisciplines

Journal Title	Two-Year Impact Factor	Five-Year Impact Factor
Nature	34.480	32.906
Science	29.747	31.052
Angewante Chemie International Edition	11.829	11.848
Journal of the American Chemical Society	8.580	8.805
Journal of Biological Chemistry	5.328	5.440
Analytical Chemistry	5.214	5.625
Journal of Physical Chemistry C	4.724	4.229
Journal of Organic Chemistry	4.219	3.994

For science education journals that publish chemistry education research articles, the two-year impact factors are all greater than 1.000. Two of the four journals have five-year impact factors approaching 3.0. However, the immediacy indices again indicate the slow pace of publication and integration of work in science education. Impact factors are not as likely to provide reliable data for making comparisons in a small field that has a slower pace of publication and integration of research. We believe that a method to supplement impact factor comparisons in determining the top-tier journals within chemistry education research needs to be explored.

The inspiration for this study and its general design originated from a mathematics education research paper *Report on Venue Study*.[12] The study focused on ranking publication venues, including peer-reviewed journals and conference proceedings, within research on undergraduate mathematics education. Questionnaires were sent out to 49 undergraduate mathematics education researchers asking them to place 22 journals in three distinct categories: Category 1 was for the most prominent venues in the field, Category 2 indicated a strong refereed venue, while Category 3 was described simply as another refereed venue. Ranking the quality of the journals in the field was an effort to provide analysis from those in the field about the quality, prestige, and influence of individual journals. This information in turn could be used to inform the promotion and tenure process.

Thus, we set out to determine how chemistry education researchers rank peer-reviewed journals in chemistry education and science education.

■ METHOD

Survey Pool

The population surveyed for this study comprised two sources. A list of chemistry education researchers was identified by using the Web site constructed by Stacey Lowery Bretz containing a list of universities that have chemistry education programs (masters and doctoral level) and the faculty who direct these programs.[13] A second source noted as "journal authors" was comprised of a list of faculty members who have published in the Research: Science and Education section in the *Journal of Chemical Education* (*JCE*) between the years 2000 and 2009 or had published a research article in *Chemistry Education Research and Practice* (*CERP*) between the years 2005 and 2009. The year 2005 marks when *University Chemistry Education* published by the Royal Society of Chemistry, and *Chemistry Education Research and Practice in Europe* originally published by the

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Table 3. Ranking and Statistical Analysis of Chemistry Education Journals

Journal Title[a] (Country of Publication)	Category 1	2	3	Total *N*	Response Statistics Mean[a]	Median	SD	Respondents, %
Journal of Chemical Education (USA)	70	20	3	93	1.28	1	0.52	86.9
Chemistry Education Research and Practice (U.K.)	53	32	6	91	1.48	1	0.62	85.0
The Chemical Educator (USA)	7	51	32	90	2.28	2	0.6	84.1
Biochemistry and Molecular Biology Education (USA)	9	32	39	80	2.38	2	0.68	74.8
Australian Journal of Education in Chemistry (AUS)	8	31	48	87	2.46	3	0.66	81.3
Education in Chemistry (U.K.)	8	25	51	84	2.51	3	0.67	78.5
Educación Química (MEX)	4	26	53	83	2.59	3	0.59	77.6

[a] The journals are ordered from lowest to highest mean values.

Table 4. Ranking and Statistical Analysis of Science Education Journals

Journal Title[a] (Country of Publication)	Category 1	2	3	Total *N*	Response Statistics Mean[a]	Median	SD	Respondents, %
Journal of Research in Science Teaching (USA)	73	14	2	89	1.20	1	0.46	83.2
International Journal of Science Education (U.K.)	64	17	8	89	1.37	1	0.65	83.2
Science Education (USA)	54	21	11	86	1.5	1	0.72	80.4
Research in Science Education (AUS)	34	36	13	83	1.75	2	0.71	77.6
Journal of Science Education and Technology (USA)	23	52	11	86	1.86	2	0.62	80.4
Journal of Science Teacher Education (USA)	16	44	21	81	2.06	2	0.68	75.7
Journal of College Science Teaching (USA)	12	49	24	85	2.14	2	0.64	79.4
School Science and Mathematics (USA)	9	32	41	82	2.39	2	0.68	76.6
The Science Educator (USA)	3	38	39	80	2.45	2	0.57	74.8
Science Education International (U.K.)	7	29	43	79	2.46	3	0.66	73.8
Canadian Journal of Science, Mathematics, and Technology Education (CAN)	1	23	53	77	2.68	3	0.50	72.0
Journal of Nano Education (USA)	4	14	62	80	2.73	3	0.55	74.8
Journal of Women and Minorities in Science and Engineering (USA)	4	11	64	79	2.76	3	0.54	73.8
Resonance: Journal of Science Education (India)	2	13	59	74	2.77	3	0.48	69.2
Journal of Baltic Science Education (Lithuania)	5	7	70	82	2.79	3	0.54	76.6

[a] The journals are ordered from lowest to highest mean values.

University of Ioannina merged to form *Chemistry Education Research and Practice* published by the Royal Society of Chemistry.

The two sources yielded an overall population of 267 faculty members. Chemistry faculty members from 32 countries representing six different continents were in the pool. It was rather United States-centric with 146 out of 267 faculty being employed at a U.S. college or university. In Europe, 66 faculty members were identified within the pool; 25 of those resided in the U.K.

Data Collection

The survey was conducted using a program that allows for the anonymous collection of data through a Web-based software interface. An invitation to complete the survey was sent through the Qualtrics software to the 267 faculty members.

The Survey

The survey was composed of three questions. The first question contained a list of seven international journals that publish chemistry education research in the United States, Mexico, the U.K., and Australia. The seven journals were selected based on frequent citation across the chemistry education literature. For each journal, faculty members were asked to mark one of three choices that ranked the quality of the journal. Category 1 indicated a top-tier journal, one of the most prominent in the field. Category 2 indicated a middle-tier journal. And, Category 3 was a low-tier journal that either chemistry education researchers were unfamiliar with or considered to be the least influential in the field. The second question in the survey asked respondents to classify 15 science education journals using the same categorization scheme. The science education journal set was selected based on frequent citation across the chemistry education literature. The third question asked the survey respondents to provide the names of any peer-reviewed journals that were not listed in the previous two questions and to classify those journals using the categorization scheme.

Survey Mailing

The survey was available for 30 days and a reminder e-mail invitation was sent 15 days after the initial invitation to those faculty members who had not completed the survey, a feature available with the Qualtrics software. All response data were downloaded at the conclusion of the 30-day data collection period and stored on a password-protected computer.

■ RESULTS AND DISCUSSION

A promising response rate of 40% was obtained (107 of the 267 participant population responded). The data were analyzed

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for each question completed by the 107 survey respondents and are discussed below. Responses and analyses for chemistry education journals are displayed in Table 3. The journals are ordered by mean from smallest to largest, with the *Journal of Chemical Education* (USA) having been ranked the highest with a mean of 1.28 and *Educación Quimica* (MEX) having been ranked lowest with a mean of 2.59. The percentage of respondents is included in the last column of Table 2 to indicate that not every journal was ranked by every participant. Calculation of mean, median, and standard deviation did not include responses left blank; therefore, blank responses did not adversely impact statistical analysis.

ANOVA methods were used to test for differences in responses for each journal by geographical location. No significant effect on rankings by geographical location of the respondent emerged.

The data showed that the journals rated by active chemistry education researchers as most prominent are the *Journal of Chemical Education*, jointly published by the ACS Division of Chemical Education and ACS journals, and *Chemical Education Research and Practice*, published by the Royal Society of Chemistry. Middle-tier journals include *The Chemical Educator* and *Biochemistry and Molecular Biology Education*. Lower-tier journals include the *Australian Journal of Education in Chemistry*, *Education in Chemistry*, and *Educación Quimica*.

Table 5. Average Rankings for *JRST*, *IJSE*, and *Science Education* for Those Who Ranked *JCE* or *CERP* as a Category 1 Journal

Chemistry Education Journal	Average for *JRST*	Average for *IJSE*	Average for *Science Education*
JCE = 1	1.15	1.33	1.55
CERP = 1	1.17	1.29	1.38

Table 6. Average Rankings for *JCE* and *CERP* for Those Who Ranked *JRST*, *IJSE*, or *Science Education* as a Category 1 Journal

Science Education Journal	Average for *JCE*	Average for *CERP*
JRST = 1	1.23	1.40
IJSE = 1	1.22	1.38
Science Education = 1	1.30	1.35

Responses and analyses for science education journals are displayed in Table 4. The four highest-ranked science education journals are indexed in the ISI and have reported impact factors. Again, ANOVA methods were used to test for differences in responses for each journal by geographical location. No significant effect on rankings by geographical location of the respondent emerged.

Upon the basis of faculty responses and the analyses, the most prominent journals were the *Journal of Research in Science Teaching*, *International Journal of Science Education*, *Science Education*, and *Research in Science Education*. Middle-tier journals were the *Journal of Science Education and Technology*, *Journal of Science Teacher Education*, *Journal of College Science Teaching*, and *School Science and Mathematics*. Lower-tier science education journals were the *Science Educator*, *Science Education International*, *Canadian Journal of Science, Mathematics, and Technology Education*, *Journal of Nano Education*, *Journal of Women and Minorities in Science and Engineering*, *Resonance: Journal of Science Education*, and *Journal of Baltic Science Education*.

It is illustrative to compare ratings of the most highly ranked journals in chemistry education—th *Journal of Chemical Education* and *Chemistry Education Research and Practice*—with those in science education: the *Journal of Research in Science Teaching* (*JRST*), the *International Journal of Science Education* (*IJSE*), and *Science Education*. All these journals have editorial boards that include members from the broad international community of researchers except for *JCE*, which has an editorial advisory board composed of faculty and chemists from the United States.

Additional evidence about consistency of respondent opinions may be explored by looking at Table 5, which displays averages for *JRST*, *IJSE*, and *Science Education* for those who ranked *JCE* or *CERP* as a Category 1 journal. Table 6 displays averages for *JCE* and *CERP* for those who ranked *JRST*, *IJSE*, or *Science Education* as a Category 1 journal.

An ANOVA was used to test for differences in mean values for the science education journals reported in Table 5. For *JCE*, significant differences were found: $F(2, 196) = 7.32$; $p = 0.001$. Tukey posthoc comparisons indicated a significant difference between the means for *JRST* and *Science Education*, $p = 0.001$. For CERP, no significant differences were found: $F(2, 150) = 1.717$; $p = 0.183$. An analysis of the means in Table 6 demonstrated significant differences between the means of *JCE* and *CERP* for *JRST* only: $t(71) = 2.045$; $p = 0.045$. The analysis of the means does not display a pattern of bias for one journal over another.

Table 7. Additional Journal Titles Supplied by Respondents with Rankings Ordered by the Number of Times Mentioned

Additional Journals (Country of Publication)	Times Listed, *N*	Category 1	Category 2	Category 3	Mean	Times Unranked, *N*
Eurasia Journal of Mathematics, Science & Technology Education (INT)	5	0	3	1	2.25	1
Science and Education (Netherlands)	3	0	3	0	3	0
School Science Review (U.K.)	3	0	2	1	2.33	0
International Journal of Science and Environmental Education (INT)	3	0	1	1	2.5	1
International Journal of Mathematics and Science (INT)	3	0	3	0	2	0
Journal of Computers in Mathematics and Science Teaching (USA)	2	0	2	0	2	0
Journal of Science & Technology Education Research (INT)	2	1	1	0	1.5	0
Research in Science and Technological Education (U.K.)	2	0	2	0	2	0
Revista Ensenanza de las Ciencias (Spain)	1	0	2	0	2	0
Studies in Science Education (INT)	1	1	0	0	1	0
Acta Didactica Napocensia (Romania)	1	0	0	1	3	0
Primary Science (U.K.)	1	0	0	1	3	0
International Journal of Science, Mathematics, and Technology Education (INT)	1	0	1	0	2	0

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Although statistically significant differences exist, overall the data demonstrate agreement among researchers on the top-tier publication venues that publish chemistry education research.

Table 7 lists the 13 additional journals supplied by survey participants. Journals are ranked by the number of times each was listed by participants. Data include the categorical rankings and means; however, it should be noted that no meaningful analysis of how these journals should be categorized can be gleaned from the low response rates for each journal. It can be concluded from the low response rates for each journal that the major journals in the fields of chemistry and science education research were included in question one and two of the survey.

■ CONCLUSION

This survey provides a method of indicating how faculty who direct and publish chemistry education research rank journals in the field. The rankings can be used by those seeking promotion and tenure as a method of identifying the most prestigious journals in the field beyond impact factor values. In the field of chemistry education research publication, venues exist that are not indexed by the ISI; thus, the results of this survey are useful for describing the standing of nonindexed journals within the field.

The results of the survey provide a snapshot of how chemistry education researchers categorize 22 chemistry and science education journals. The top-tier chemistry and science education journals based upon this survey are the *Journal of Chemical Education*, *Chemistry Education Research and Practice*, the *Journal of Research in Science Teaching*, the *International Journal of Science Education*, and *Science Education*. All of these journals are indexed by the ISI.

We believe that the results of this survey are not static and that the survey should be repeated every two to five years as opinions of the quality of these journals can change over time as new faculty become engaged in the field. New journals that publish either chemistry or science education research could also emerge, necessitating their inclusion in a subsequent survey.

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ARTICLE

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Examining the Relationships among Doctoral Completion Time, Gender, and Future Salary Prospects for Physical Scientists

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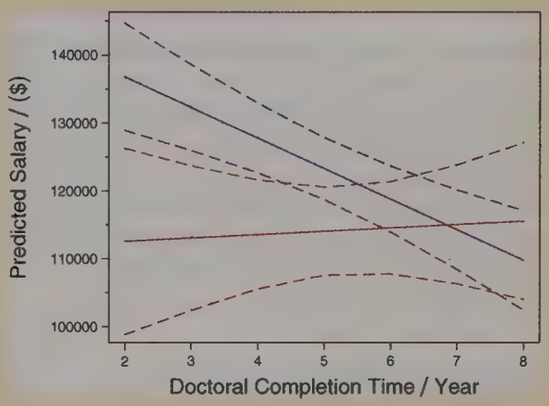
ABSTRACT: Using data from a national survey of Ph.D.-holding chemists and physicists, time-to-doctoral degree is found to be a strong predictor of salary: each additional year in graduate school corresponds to a significantly lower average salary. This is true even while controlling for standard measures of scientific merit (grant funding and publication rates) and several other factors expected to influence salaries (field of research, type of position and rank, type of employing institution, years of seniority, and age). This picture is complicated by the inclusion of gender in the analysis, which reveals that women earn significantly less than men overall and experience no effect of doctoral completion time on their salaries, while men do see a significant gain in salary stemming from earlier completion times. Further investigation indicates that doctoral completion time is largely unconnected to measures of prior academic success, research independence, and scientific merit. This suggests that doctoral completion time is, to a great extent, out of the control of individual graduate students. Nonetheless, it can be influential on an individual's future career prospects, as can gender-related effects.



KEYWORDS: Graduate Education/Research, Chemical Education Research, Testing/Assessment, Student/Career Counseling, Women in Chemistry

INTRODUCTION

Many years ago, in their book *Becoming Professional*, Bucher and Stelling provided a moral compass for graduate educators, writing (ref 1, p 280):

> [I]f, as we think, it is important that individuals retain some control over their own destinies, then it behooves the trainers to inform potential trainees about the probable consequences of going through any particular training program and to give them as much information as possible about the nature of passage through that program.

In order to meet this challenge and, concurrently, identify areas for reform in graduate education, it is necessary to understand the relationships between graduate school experiences, measures of scientific success, and career outcomes. In particular, one would like to know how success is measured for a doctoral student in the physical sciences. Unlike other levels of education where degree and diploma attainment relies on relatively structured assessments (tests and exams, rubrics, etc.), a doctoral degree relies more heavily on unstructured assessments of research. This ambiguity is inextricably linked to the unique goal of graduate-level science education: the training and development of scientific researchers who have specialized talents and research agendas. By definition, doctoral research should consist of an original investigation that explores a previously unanswered problem. Every doctoral student experiences a high degree of uniqueness in their graduate program. So appropriate measures with which to evaluate individuals are less obvious than at other levels of education.

Despite these essential differences between graduate school and other levels of education, there are certain canonical qualities and skills that a doctorate is supposed to signify: the ability to define a research problem, develop or learn a methodology to appropriately explore the problem, carry out the necessary experimentation and, ultimately, discover answers to the problem. An ideal graduate student develops into one of the "symbolic–analytic workers" of science:[2] those individuals who are responsible for "the problem-solving, -identifying, and strategic-brokering activities" (ref 3, p 177) of the scientific endeavor, including the requisite synthesizing, assessment, and systematizing of research. It is these people who set the agenda for future research: they form the core of the peer-review system, determining which problems have worth, which should be funded, who should be supported and encouraged to carry out certain research and, ultimately, who has merit in the scientific endeavor.

How, then, are graduate students evaluated as they develop into full-fledged members of the scientific community? Possible indicators include grades, GPA, and GRE scores. An earlier meta-analysis[4] found a small correlation[5] ($r \sim 0.07$) between Ph.D.-level

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GPA and "job performance", noting that "the work of many Ph.D.s and MDs is difficult to measure and admission to such programs is more selective than undergraduate programs." (ref 4, p 552) Moreover, correlations between GPA and job performance were found to be lower in the sciences than in other fields. More recently, Kuncel and Hezlett completed a meta-analysis to find that standardized test scores are "valid predictors of many aspects of student success across academic and applied fields" (ref 6, p 1080); however, the correlation between such scores and the most scientifically relevant outcomes, including citation counts and research productivity, are notably smaller[5] (~0.1−0.2) than short-term success outcomes such as first-year graduate GPA, overall graduate GPA, and qualifying exams (~0.4−0.5).

Another possible measure of a graduate student's scientific merit is doctoral completion time. However, there are a number of concerns about the appropriate interpretation of doctoral completion time. In the social sciences, Picciano et al,[7] reported that graduates' self-reported evaluation of program quality, quality of professional training, career goals, and satisfaction with their dissertation advisors all had significant impacts on time-to-degree. The authors concluded that time-to-degree could, to some extent, be interpreted as a measure of the quality of doctoral preparation, but cautioned that "[S]tudents with more ambitious projects and programs that support riskier endeavors might have longer average [times-to-degree], but should not be discouraged simply because of this" (ref 7, p 8).

It has been noted that doctoral completion times in many fields have increased in recent decades. In the physical sciences, the median time-to-degree has increased from 5.9 to 6.8 years in the 25-year period between 1978 and 2003.[8] Other research has indicated that a number of factors can influence time-to-doctoral-degree, including financial support,[9] to some extent, gender,[10] and disciplinary and departmental issues.[11−14] In particular, doctoral completion time has a convoluted relationship with the quality of a doctoral program, as the former has often been used, in part or in whole, as a measure of the latter.[15,16] The finding that many independent factors have a significant impact on time-to-degree suggests that doctoral completion time is a complex and possibly muddled measure of scientific merit.

In a multidisciplinary study, Lovitts studied doctoral completion and attrition across several graduate departments.[11] The author noted that some graduate educators have implicitly assumed that a student deficiency model could explain doctoral completion and attrition; that is, it has often been assumed that weaknesses or deficiencies found in students' backgrounds lead to a failure of the timely completion of doctorates. Instead, Lovitts found that two particular departmental factors explained a significant amount of the variance in student attrition rates; namely, the effort and resources a department commits to the integration of graduate students and the efforts made toward students' development and understanding of the formal and informal structures of their graduate programs and departmental research cultures.

Fox has pointed out that "[C]ompared with nonscientific fields, sciences are fundamentally 'social' and 'organizational'" (ref 7, p 658), emphasizing the importance of social interaction and culture to the workings of graduate science education. In particular, she noted their potential impacts on women scientists. The former finding is reinforced by the findings of Golde,[12−14] who has identified several disciplinary and departmental themes that explain doctoral completion including: mismatched research practices and student strengths; mismatched expectations between students and departments; structural isolation of students; student perceptions of a poor job market upon graduation; and problems of advisor−advisee compatibility.

These issues are particularly relevant to the physical sciences, which have suffered from a lack of growth for at least four decades and have shown consistently high rates of attrition.[18,19] Furthermore, as mentioned earlier, doctoral education is becoming longer,[8] which implies that pursuing a doctorate is an increasingly large commitment, both for individuals and the graduate programs and universities who need to support them. Thus, understanding doctoral completion time and the long-term impacts of graduate experiences could shed some light on these problems.

The continued dearth of women in chemistry and, especially, physics at the graduate level and beyond is well-known.[19] The National Research Council has long studied issues related to women in STEM, with mixed findings.[20−22] In 2001, the NRC reported that gender differences in employment across various sectors in STEM had shrunk substantively in the period 1973−1995 noting that, in institutions ranked as R-I by the Carnegie foundation (a ranking system that was abandoned after 1994 and is no longer advocated by Carnegie, though still widely known), "[M]en and women have become increasingly similar in their distribution among types of institution" (ref 20, p 6). In terms of educational preparation, it also noted significant declines in differences between men and women on many characteristics of their educational background, including ranking of their Ph.D. institutions and their doctoral completion time. However, this report also found that substantial differences between men and women persisted, including gaps in representation and salary differences—salaries for women appeared to become stagnant after about 20 years of experience, while men's salaries continued to rise. In 2010, again using survey data collected from faculty in a set of Carnegie-ranked R-I institutions, the NRC reported that women and men appeared to have "[e]njoyed comparable opportunities within the university, and gender does not appear to have been a factor in a number of important career transitions and outcomes" (ref 22, p 4). Despite this, substantial gaps in the representation of male and female faculty persisted. In their sample, only 24.1% of assistant professors and 7.6% of full professors of physics were women, a difference assigned to attrition at the critical transition points in career development.

To summarize, an outstanding issue from the existing research on doctoral education is whether or not doctoral completion time should be thought of as a faithful measure of scientific merit. If more dedicated and capable students ("go-getters") finish more quickly, then perhaps there is some value in employing doctoral completion time as a measure of potential and capability upon graduation. However, if students with more ambitious dissertation research projects tend to finish later, then completion time is a less optimal standard for assessing potential and pushing students to finish faster could diminish their opportunities to carry out truly influential research or lower graduate education standards. As such, this study first explores the impact that doctoral completion times have on graduate students' future salary prospects, then examines whether doctoral completion times are actually predicted by measures of merit, and, third, whether there are gender-specific effects present in either doctoral completion times or career prospects. The research questions that are addressed are:

- What are the long-term impacts, if any, of doctoral completion time on chemists' and physicists' employment prospects, as measured by their postgraduate salaries?

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- What factors influence doctoral completion time in chemistry and physics?
- Are there gender-specific effects that influence doctoral completion time or physical scientists' postgraduate salaries?

To address these questions, a set of analyses was undertaken to explore them quantitatively. This study goes beyond previous work, which has often focused on R-I academic institutions, by considering physical scientists in several sectors, including industry, government, and across academia.

METHODOLOGY

The data used in this paper were obtained as part of Project Crossover (NSF #0440002), a mixed methodological study designed to study the transition from graduate student to independent researcher in chemistry and physics. The first part of Crossover consisted of a set of qualitative interviews with chemists and physicists across the United States. Between 2005 and 2006, a total of 125 semistructured, open-ended interviews were conducted with various members of the chemistry and physics communities: graduate students, postdoctoral fellows, professors at all academic ranks (including two past Nobel Prize winners), industrial and other research scientists, and some individuals who had left scientific research entirely (before or after completing their doctorates).

Following the interviews, a pair of surveys for members of two professional scientific societies for physical scientists were developed based on the interview results and a review of the prior literature in this area. One survey was designed for student members of these societies; one was designed for full members. In this paper, only the data collected from the full members are analyzed. The final version of this survey included 145 questions covering a wide range of topics: demographic questions, early science interests, academic history, undergraduate and graduate school experiences, and postgraduate experiences.

Once the survey was developed, a randomized list of 13,000 physical scientists was obtained. These individuals were mailed paper versions of the survey in June 2007. They had two options for responding: by returning the paper survey in a provided envelope, or by filling out the survey online (which included entering an individualized serial number from the mailed materials to account for respondents). Four reminders were sent to those individuals who had not yet responded over a period of six months following the initial mailing. From the initial list, 557 were determined to have contained nondeliverable addresses (out of date, etc.), and 3100 were determined to have been sent to inapplicable individuals (individuals who had never participated in graduate education, student members who were incorrectly listed as full members, etc.). The number of qualified individuals with correct mailing addresses who comprised the original sample was therefore determined to be 9343. In total, 3220 completed surveys were obtained, consisting of 2136 filled out by chemists (64% online responses, 36% paper) and 1084 by physicists (60% online, 40% paper). The gender representation of the respondents was 29% female overall (29% of chemists, 31% of physicists). To assess the representativeness of the sample, respondents' demographic backgrounds (race—ethnicity and gender) and employing institution type (universities, federal agencies, nonprofit, for-profit, and other employment not fitting these categories) were compared with the National Science Foundation's WebCASPAR database.[23] The Crossover sample was found to have similar proportionate representation across these measures as the WebCASPAR data. Note also that only individuals who indicated that their doctoral programs were located inside the United States were included in the analyses that follow.

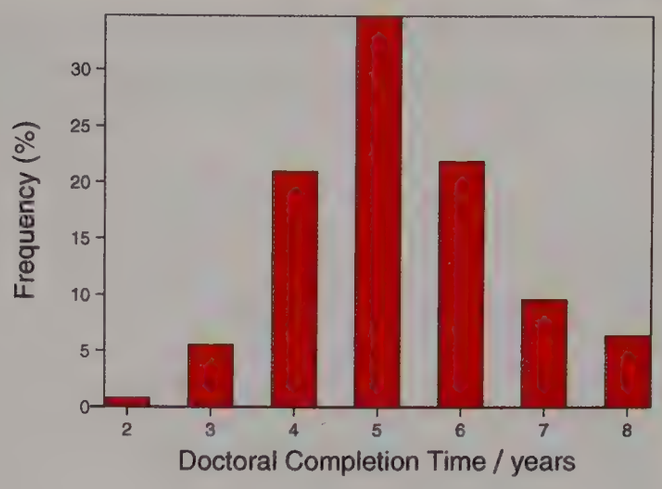

Figure 1. Distribution of doctoral completion times. The mean is estimated at 5.26 ± 1.27 years. Respondents who indicated "<3" years are weighted as two years; those who indicated "≥8" years are weighted as eight years.

RESULTS

Respondents were asked the question "From the time you first enrolled in a doctoral program, how many years did it take you to complete your Ph.D.? (Please round to the nearest year.)" The available responses were: <3, 3, 4, 5, 6, 7, ≥8. In the current analysis, respondents who indicated "<3" were weighted as two years and those who indicated "≥8" were weighted as eight years. With this choice, the mean time to complete a doctorate was found to be approximately 5.26 ± 1.27 years, similar to historic data.[8] Frequencies in the responses to this question are summarized in Figure 1. The other outcome variable used throughout this paper is individuals' responses to the item: "Fill in the dollar amount closest to your total annual income (in thousands US$)." The available responses were binned in $10,000 increments from zero up to $200,000. Respondents who indicated ">$200,000" were weighted as $210,000 throughout this analysis. With this weighting, the mean salary is approximately $101,200 ± $48,200, consistent with other national data.[24]

All statistical tests were carried out using R, a free software system for statistical computing,[25] in particular the "QuantPsyc" and "car" packages for certain functions.[26,27] Because the sample size was large, to reduce the chances of Type I error (i.e., a false positive), a stringent α cutoff of 0.01 (or 1% chance of Type I error) was set.

The Relationship between Salary and Doctoral Completion Time

First, a multiple regression model predicting individuals' current annual salary was fit. That is, we determined a model treating annual salary as a dependent variable and tested for linear relationships between this outcome and other independent variables. One feature of this approach is that multiple predictors can be simultaneously taken into consideration, allowing for multiple effects to be seen, unlike simpler analyses. See Table 1 for a summary of the resulting model, which accounts for 47.6% of the variance (as estimated by the adjusted R^2). A number of expected control variables were significant, including: type of position (full professor, associate professor, industrial or research

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Table 1. Results of the Multiple Regression Model with Outcome of Salary

Parameters[a]	B, $[a]	SE, $[b]	β[c]	Significance
Intercept	98,840	6090	—	<0.001
Controls:				
Position		Included		
Field		Included		
Institution		Included		
Years of Seniority		Included		
Age		Included		
Marital Status		Included		
Citizenship Status		Included		
Time Lapse between UG and Grad Enrollment		Included		
Grant Funding (per $10,000 of grant funding)	120	10	0.197	<0.001
Publications (any authorship, per publication)	80	20	0.120	<0.001
Primary/1st Author Publications (per publication)	110	30	0.079	0.001
Ph.D. Completion Time (per year)	−3370	700	−0.090	<0.001
R^2 (Adjusted R^2)		0.485(0.476)		
N		1740		

[a] Values in this column indicate the estimated regression coefficients (unstandardized). [b] SE indicates the standard error associated to each regression coefficient. [c] β indicates the standardized regression coefficients.

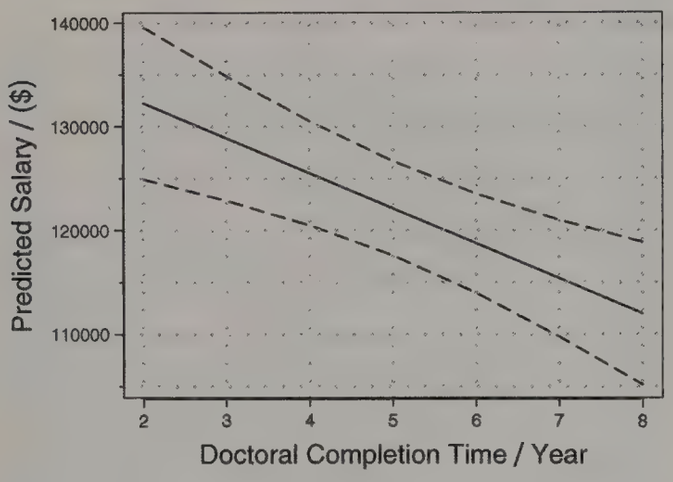

Figure 2. Predicted salary plotted with varying doctoral completion time. Confidence intervals (99%) are indicated by the dashed lines. All other factors appearing in Table 1 are held to their median values.

scientist, postdoctoral researcher, etc.), and type of employing institution (public academic, private academic, industry, government-funded laboratory, etc.).

Clearly, individuals' current occupation and the type of the employing institutions have a significant impact upon their salaries, and so were input into the regression. Owing to the complexity of participants accurately identifying Carnegie classifications (or the now-defunct rankings) of their doctoral institutions and employing academic institutions, it was not possible to collect this information. Furthermore, a large portion of the sample was composed of industrial and research scientists for whom this type of information is not applicable to their employer. Thus, by identifying broad categories of current occupation and currently employing institution, we could model, to a great extent, the variance in salary arising from postgraduate career choices. The following background factors were also included in the model: field of research (chemistry or physics), number of years of seniority, age, marital status, citizenship status, and time lapse between undergraduate and graduate enrollment. For brevity, the regression coefficients for the many control variables are omitted from Table 1.

Three measures of scientific productivity were found to be correlated with salaries: total grant funding received ($p < 0.001$), total number of publications (any level of authorship, $p < 0.001$), and total number of primary or first-author publications ($p = 0.001$). With respect to grant funding, for every $10,000 received in their career, a scientist earns an extra $120 per year, on average. With respect to publications, a scientist earns $80 dollars for every additional publication (any level of authorship) and a further $110 if she or he was the primary or first author.

Interestingly, even with all the aforementioned factors included in the model, an individual's time-to-doctoral-degree is a significant predictor of current salary: longer doctorates correspond to lower salaries, by $3370 per year spent in a doctoral program ($p < 0.001$). See Figure 2 for a graphical representation of this effect. Thus, a scientist who indicated that he or she completed the doctorate in four years earns, on average, approximately $10,000 more each year than one who spent seven years in their doctoral program, even if that scientist is equivalent on all other measures included in the regression model. The size and significance of this effect, showing that doctoral completion times are strongly correlated with future salary prospects, suggests that time-to-degree might be (or at least be interpreted as) an indicator of scientific merit. To investigate this possibility, doctoral completion time was investigated next.

Modeling Doctoral Completion Time

A second multiple regression model was then fit on doctoral completion time. See Table 2 for a summary. Several factors were found to be significant predictors of doctoral completion time. The first set of variables relate to departmental or programmatic factors. Physicists take, on average, 0.50 years longer to finish than chemists ($p < 0.001$). Students who were required to take courses over a greater number of academic years spent more time completing their doctorates, by 0.25 years per year of coursework ($p < 0.001$). Those who taught more during graduate school took slightly longer, by

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Table 2. Results of the Multiple Regression Model with Outcome of Time to Doctoral Degree

Parameters[a]	B, Years[a]	SE, Years[b]	β[c]	Significance
Intercept	5.11	0.17	—	<0.001
Field (Physics is longer)	0.50	0.06	0.189	<0.001
Years of Required Coursework (per year)	0.25	0.04	0.151	<0.001
Number of Courses Taught during Grad. School (per course)	0.04	0.01	0.140	<0.001
Age	−0.014	0.002	−0.144	<0.001
Took Any Part of Comps More Than Once	0.24	0.07	0.074	<0.001
Timing of Choice of Dissertation Topic (20-point time scale)	0.07	0.01	0.114	<0.001
Dissertation Topic Changed Significantly	0.27	0.08	0.089	<0.001
Dissertation Topic Changed Completely	0.32	0.10	0.072	0.002
MS Awarded upon Leaving Another Program	0.50	0.13	0.077	<0.001
Satisfaction with Advisor (4-point scale)	−0.20	0.03	−0.138	<0.001
Changed Doctoral Advisors	0.32	0.08	0.081	<0.001
R^2 (Adjusted R^2)		0.206(0.199)		
N		1880		

[a] Values in this column indicate the estimated regression coefficients (unstandardized). [b] SE indicates the standard error associated to each regression coefficient. [c] β indicates the standardized regression coefficients.

0.04 years per course taught ($p < 0.001$). Another predictor confirmed that doctoral programs are becoming, on average, longer: younger individuals had longer doctorates, by 0.014 years per birth year ($p < 0.001$), echoing the findings of Hoffer and Welch.[8] Thus, according to the model, an individual in this sample who completed his or her doctorate 30 years ago would have taken, on average, 0.42 fewer years than an individual completing a doctorate today.

A few factors related to an individual's personal development and research progress were found to be significant. If an individual indicated that she or he had to take any part of the comprehensive or qualifying exams more than once, that person spent an average of 0.24 extra years in graduate school ($p < 0.001$). If individuals indicated that they decided on their dissertation topic later in their program, they took longer to complete their doctorate (0.07 years per point on a 20-point time scale, $p < 0.001$). Furthermore, if an individual indicated that the topic of her or his dissertation changed significantly or completely from the start to the end of their doctorate, then the completion time was longer by 0.27 years ($p < 0.001$) or 0.32 years ($p = 0.002$), respectively, than those whose final dissertation topic was identical to the one with which they started.

There was one significant factor related to students' prior graduate school experiences: individuals who had received a Master's degree upon leaving a previous doctoral program took 0.50 years longer to complete their doctorates ($p < 0.001$). This factor may be identifying individuals who experienced particular struggle in graduate school (and, yet, persevered through another doctoral program) or those individuals who had significantly changed their research areas or career plans after beginning an earlier doctoral program.

There were two significant predictors specifically related to the advisor–advisee relationship. Respondents were asked to rate, on a four-point scale, their level of satisfaction with their professional relationship with their dissertation advisor. Individuals who were more satisfied had shorter doctoral programs (by 0.20 years per point on the scale, $p < 0.001$), similar to the findings of Picciano et al.[7] In addition, if a student reported that he or she changed dissertation advisors during the program, then he or she spent an average of 0.32 extra years in a doctoral program ($p < 0.001$).

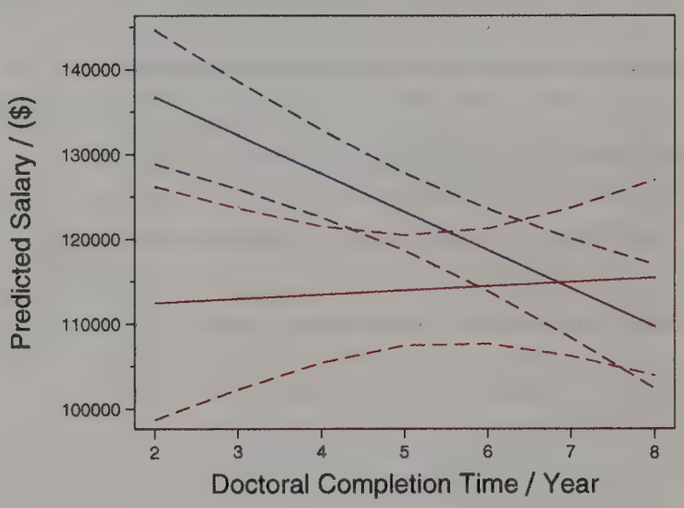

Figure 3. Predicted salary plotted separately for men and women with varying doctoral completion time. Women are indicated in red, men are indicated in blue. Dashed lines indicate 99% confidence intervals.

As already mentioned, it was not possible to capture detailed data on the Carnegie classification of the doctoral institutions that individuals attended, which might provide additional insight to this model. It might be expected that students enrolled in more highly ranked institutions have shorter doctorates, and thus, Carnegie classification (or other proxies for doctoral program quality) might be expected to be a significant predictor of doctoral completion time. We did test several related measures: for example, students' prior grades (both in high school and undergraduate institution) might be higher for students enrolled in more highly ranked institutions (because of higher admissions standards), thus, leading to shorter doctorates. In fact, these were found to be nonsignificant predictors of doctoral completion time.

Exploring Gender Effects in Salaries and Doctoral Completion Times

Extending the models appearing in Tables 1 and 2 to consider possible gender effects reveals some additional, interesting results. When included in the model predicting salary, gender was found to be a significant predictor ($p < 0.001$), in that women earn

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significantly less than men, all other factors being held equal. This effect is supported by the findings of Lovitts[11] who also found that gender was a significant predictor of future salary, with women earning less than men, an effect that appeared to grow as time increased after the end of the doctoral experience. In the current data, a significant interaction also emerged between gender and doctoral completion time, which partly ameliorates the effects of time-to-degree, though it does not entirely account for the salary differences of men and women ($p = 0.002$).

The combined effects of the gender and gender−completion time interaction factors are displayed in Figure 3. For men, the effect identified earlier is still true: males who finish faster earn significantly greater salaries. For women, the plot of salary versus completion time is, in fact, flat but located well below the plot for men. As can be seen graphically, the net effect of gender and doctoral completion time is such that women earn significantly less money than men who finish relatively quickly (five years or fewer), but there is little difference in salary between men and women who finish in seven or more years. These results suggest that while doctoral completion time has a large impact on future salary prospects for men, gender-related effects have an even greater impact on women scientists. Recall that survey respondents' age, marital status, rank, and seniority have been controlled for in this model, so the gender gap cannot be explained by differences in these factors. At first glance, this result appears to be somewhat at odds with recent National Research Council findings[22] reporting similar salaries for women and men in academic chemistry and physics. However, the sample used was somewhat small (N = 198 and 216, respectively), skewed in its gender representativeness (47% and 51%, respectively, of respondents were women—far higher than their overall presence in these populations), and, most importantly, were taken solely from R-I institutions. Our data are more representative of the national population of Ph.D.-holding chemists and physicists, which includes many individuals who are not in tenure-track or tenured positions in academic institutions, so the NRC findings do not appear to be in contradiction with our own.

Note also that we considered several other interaction effects in the model predicting salary. Specifically, we tested for interactions between gender and the occupational category and the type of employing institution, respectively. If we found significant gender interactions in either of these categories, then it might provide evidence that the apparent differences in salary between men and women are due to women being employed in substantially different, lower-paying types of jobs or institutions. In fact, interactions between gender and occupational category and type of employing institution were found to be nonsignificant except for one minor case: women employed at government-funded laboratories experience a smaller gap in salary in relation to their male counterparts ($p = 0.009$) than other cases (though they still earn less, on average). Thus, these results do not support this alternative hypothesis that the gender gap in pay is due to systematic differences between men and women in occupation or employer type.

Including gender in the model predicting doctoral completion time reveals that gender is not a predictor of doctoral completion time. Men and women finish, on average, in the same number of years. This result is generally consistent with other work.[17]

Taken together, the result of including gender effects in the two models suggests that women perform equivalently to men through the end of graduate school, as evidenced by the lack of gender effects in doctoral completion time. Nonetheless, after graduation, the salary prospects of men and women clearly follow a different pattern. Women earn the same (lower) salary regardless of their time-to-degree while men who finish faster earn significantly more.

■ DISCUSSION

Reconsidering the Link between Doctoral Completion Time and Future Salary

One possible explanation for the overall relationship between doctoral completion time and future salaries is that individuals who finish faster are more scientifically meritorious. However, the gender effects that are shown in Figure 3 go directly against the hypothesis that doctoral completion time is an appropriate measure of merit; otherwise, one would not expect there to be a different effect for women and men. Furthermore, the regression model predicting completion time provides little evidence to support the merit hypothesis. Many variables were considered in constructing the model in Table 2 that could have provided support for this idea, but, nonetheless, were found to be nonsignificant. For example, variables representing previous grades, including high school and undergraduate studies, were found to be nonsignificant. Moreover, individuals' motivations for entering graduate school and early interests in science,which has been found elsewhere to be an indicator of actual scientific productivity,[28] were also unrelated to doctoral completion time. Third, respondents were asked to evaluate their level of research independence by the end of their graduate programs (on a four-point Likert-type scale ranging from "Completely depended on collaborators" to "Used only my own ideas") but this, too, was not a significant predictor. By and large, doctoral completion time appears to be variant on programmatic and advisor-related factors not directly connected to individuals' abilities or scientific merit.

With all these results in mind, then, it is worthwhile to consider the mechanisms through which the connection between doctoral completion time, gender, and postgraduate salaries could be manifested. At least three possible mechanisms exist: doctoral advisors' support; students' expectations; or potential employers' level of interest. Though the current data set cannot definitively answer these questions, it is worth considering how each mechanism could affect individuals. In the first case, advisors may feel that certain students (those who take longer to complete, women, etc.) are less scientifically gifted and, therefore, are less apt to help these students find the best-paying positions or to give the highest level of all-around support and encouragement. In the second case, students with lower expectations (stemming from spending extra years in graduate school or from affective factors, such as depressed self-efficacy or science identity) may be more apt to accept a position of lower salary, apply to certain types of postgraduate positions, or to negotiate less aggressively.[29−32] In the third case, potential employers or hiring committees may, if they have the opportunity, pass over students who finish later in favor of students who finish earlier, at least with respect to male scientists.

For all three of these scenarios, each of which is likely to be relevant at various times to the postgraduate job searches of Ph.D.-holding individuals, a commonality is the understated assumption of connections between doctoral completion time and scientific merit on the one hand, and gender-related issues and scientific merit on the other hand.[33] Hence, certain students—those who take longer to complete their doctorates or some

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women—are assumed (by their advisors, themselves, or potential employers) to have less scientific potential. But, again, the analysis presented here indicates that there is little to no empirical evidence to support these assumptions.

■ CONCLUSIONS AND FUTURE WORK

To return to the central questions that were pursued in this paper, doctoral completion time is a significant predictor of the overall career prospects of physical scientists, as measured by their future salary. This picture is complicated by the inclusion of gender into the analysis, which shows (as in Figure 3) that women, regardless of doctoral completion time, earn about the same salary, which is at the low end of the scale for men.

While salary is certainly not a comprehensive measure of career success, it is one of the main chips on the bargaining table between scientific institutions and researchers. Recall that this analysis showed that grant funding and publication rates are positively correlated with salary, indicating that the latter is a partial proxy for scientific merit. One reason why this work is important is because many currently enrolled students, advisors, and graduate programs may be unaware of the impacts of doctoral completion time and gender-related issues on future careers. As the initial quote by Bucher and Stelling[1] emphasizes, the scientific community has a responsibility at the very least to inform students of the future consequences of their choices, if not to improve negative practices.

The second model fit on doctoral completion time indicates that, while there is a significant amount of variance in time-to-degree, many of the factors to which it is significantly related are institutional or departmental in nature (e.g., teaching loads and coursework). While a few significant factors are possibly related to individuals' scientific progress in graduate school (e.g., changing dissertation topics), the bulk of the indicators related to doctoral students' abilities or aptitudes (e.g., prior academic performance, reported level of research independence) are not predictors. Recall also that an individual's motivations for going to graduate school are not significant predictors of time-to-degree, though they do predict future research productivity,[28] which implies that the sort of "go-getters" who turn out to be the most productive scientists do not necessarily finish faster.

One might wonder what other factors not captured in this analysis might explain the variance in doctoral completion time. It is likely that there are other research group, institutional, and departmental effects. In particular, the overall quality of a doctoral program might influence doctoral completion time, which could also impact future salary prospects as students from more highly ranked institutions finish faster and, simultaneously, experience better employment prospects owing to their graduate pedigree. Though it is a limitation of the current study that our data cannot model this completely, we had some proxies for doctoral program quality (such as students' prior academic success) but did not see significant effects on completion time or on salary. Furthermore, the gender effects also undermine this hypothesis: if this were the case, we would expect women to also experience increased salary from decreased doctoral completion time, which they do not.

Another source of variance in doctoral completion time is the existence of research group structures that measure, accelerate, and impede doctoral progress. During the interview phase of Project Crossover, many doctoral advisors indicated that they used personal, idiosyncratic measures to determine when their students were "ready" to defend their dissertations. For example, a student might be required to publish a minimum number of papers or to train their "replacements". These requirements also have the dual purpose of providing important, tangible research contributions to either or both the group and dissertation advisor. Depending on how these requirements are handled, they may act as a lever for speeding up the graduate process or act as a barrier to completion. The survey data could not capture sufficient detail on these issues, but they are undoubtedly a source of some of the variance in doctoral completion time.

Furthermore, it is clear that a substantial source of variance, as noted by Picciano et al.,[7] is the specific choice of dissertation research topic. Certain research areas are riskier by nature, while others have more clearly defined methodologies and research questions. Undoubtedly, the impact of these aspects of research will be significant on completion time, though it is difficult to categorize and capture the large variance found in these effects statistically. The inescapable reality that certain lines of research will result in longer completion times underlines the importance of recognizing that completion time is not a particularly worthy measure by which to compare students upon graduation.

Another important issue to note is that gender is not a significant predictor of doctoral completion time, unlike the earlier findings of Baker;[10] nonetheless, it clearly affects future salary prospects. Gender is a significant (and somewhat large) predictor of salary, echoing the finding of Fox that (ref 17, p 657)

> [A]cross science fields, the general pattern is one of similarity in doctoral origins for men and women. Gender equity in scientific careers is not a matter of simply improving the doctoral origins of women.

The causal mechanism for the gender gap in salary may fall along the lines discussed earlier: students' varying expectations, advisors' varying level of career support, or potential employers' bias. Again, determining the causes of these effects goes beyond the data analyzed here but more investigation is clearly warranted.

One of the primary reasons that Project Crossover was undertaken was in light of the general lack of research on graduate physical science education and to begin to systematically investigate some of the long-term effects of the graduate educational experience. The dearth of research in this area is due partly to the complexities of the less-structured nature of science education at this level, as well as the elite nature of graduate education: few students will ever get to experience graduate-level chemistry or physics. Nonetheless, understanding scientific practices at this level and improving upon longstanding, though not necessarily optimal, practices could have important future impacts on these fundamental research areas. Changing the focus of graduate programs and scientists away from factors that may unjustly reduce the perceived potential of new researchers could open new avenues for research that would otherwise be deemed too risky, improve the retention and persistence of women scientists, and improve the quality of research in these fields.

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ARTICLE

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Design and Implementation of a Self-Directed Stereochemistry Lesson Using Embedded Virtual Three-Dimensional Images in a Portable Document Format

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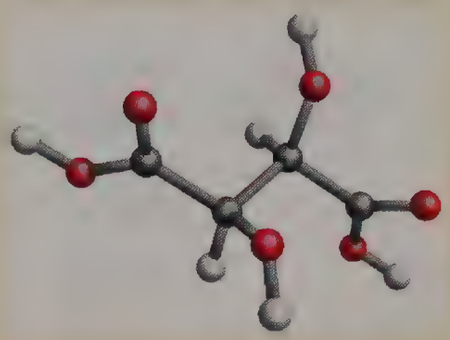
Supporting Information

ABSTRACT: A novel stereochemistry lesson was prepared that incorporated both handheld molecular models and embedded virtual three-dimensional (3D) images. The images are fully interactive and eye-catching for the students; methods for preparing 3D molecular images in Adobe Acrobat are included. The lesson was designed and implemented to showcase the 3D virtual images and determine whether the use of virtual 3D images should be permanently included with the stereochemistry lesson. A group of students completed the lesson and provided positive feedback on the incorporation of the embedded virtual 3D images.



KEYWORDS: Second-Year Undergraduate, Curriculum, Organic Chemistry, Computer-Based Learning, Hands-On Learning/Manipulatives, Multimedia-Based Learning, Enantiomers, Stereochemistry

Chemistry is often brought to life by viewing three-dimensional (3D) images of molecules using a computer. Computer visualization has been available for over a decade, but this often involves added software expense for programs such as Spartan; even when these concepts can be communicated with freeware, such as ChemSketch or Jmol, there is a significant learning curve for the software, both for the instructor and the student. Furthermore, the freeware products provide only community support. To circumvent these problems and provide universal access to 3D chemical models, virtual 3D images can now be embedded in the portable document format (pdf); these 3D images can then be viewed with the latest version of the freely available Acrobat Reader,[1] arguably the most ubiquitous digital imaging format. The use of this technology in chemical education has great promise for delivering learning in a format that is familiar and comfortable to this generation of students.

Visualization has been recognized as an important component for learning chemistry for many years;[2,3] this is especially true for the study of stereochemistry. The extensive variety of ideas and articles on teaching stereochemistry indicate the complexity and importance of the subject matter. Articles have been published about using different techniques to teach stereochemistry such as drawing,[2] transparent sheets,[3] word games,[4] one's hands,[5] and stick figures,[6] as well as the popular Darling molecular models. The challenge of teaching stereochemistry results in part from the limited ability of some students to visualize molecular structures in three dimensions.

A series of 3D pdf files have been created that can be used for teaching and assessing learning of stereochemistry in an organic chemistry sequence for majors. The virtual 3D molecular structure images may be viewed as ball and stick, wire, or spacefill. When the students view interactive 3D structures, they can rotate, spin, pan, zoom, walk, fly, and return to the default setting (see Figure 1 for a screen shot). In this article, a description of how the interactive sets of 3D molecular structures in the pdf format were created and the results of the experience in the classroom are presented.

METHODS FOR GENERATING EMBEDDED VIRTUAL THREE-DIMENSIONAL IMAGES

Creating the documents with embedded virtual 3D images may be accomplished by using the following procedures (Figure 2 and Additional Note[a]).

1. Using either ACD ChemSketch 11.0 Freeware[7] or CambridgeSoft ChemDraw,[8] the required two-dimensional (2D) structures were drawn.
2. The 2D structure was then optimized into 3D with the 3D conversion tools found in ChemSketch.
3. The 3D molecules were saved to the MDL molfile (*.mol) format. The files were then converted from a mol format to a pdb (Protein Data Bank) format, which is the format recognized by the Adobe software, using Open Babel Graphical User Interface v2.2.0[9] with the default settings.
4. Three views (wire, balls and sticks, and spacefill) of the 3D molecule were then prepared using Adobe Acrobat 3D Toolkit 8.1.0.
5. A pdf template was opened with Adobe 3D and the virtual 3D images were added using the 3D tool.
6. Often it was desirable to match the interactive 3D image with a traditional 2D static image. The addition of 2D

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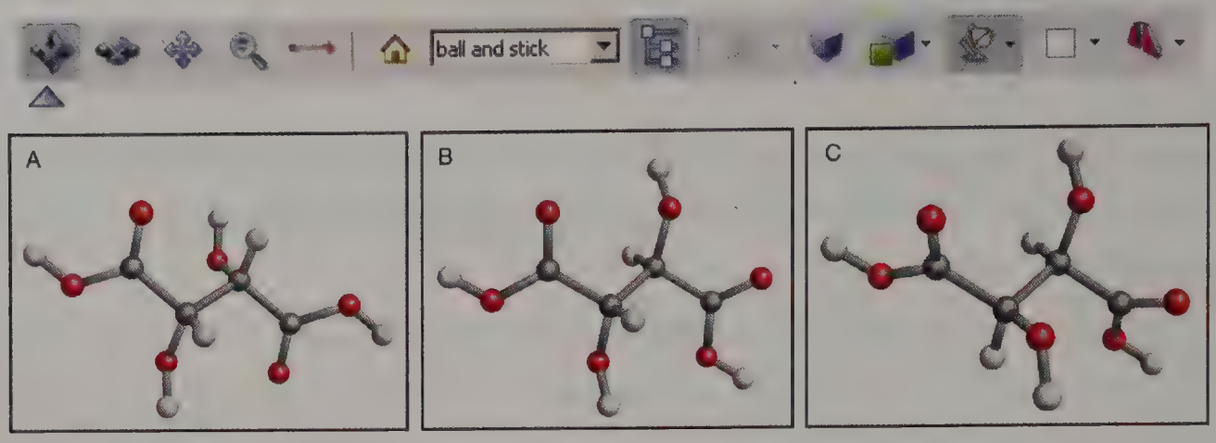

Figure 1. Screen shots of a ball and stick representation of (A) D-(−)-tartaric acid, (B) *meso*-tartaric acid, and (C) L-(+)-tartaric acid. Key: C is black, O is red, and H is white.

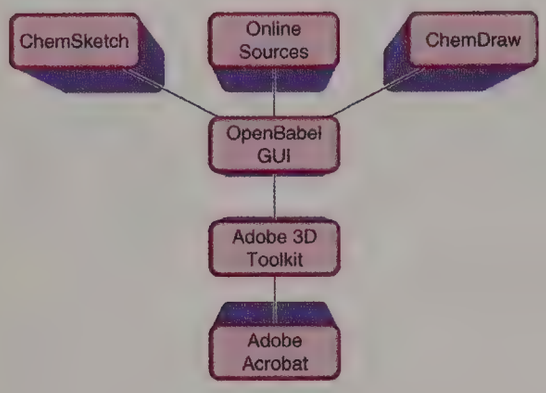

Figure 2. A general schematic showing the processes for embedding virtual three-dimensional images in pdf's.

images was completed by opening the ChemSketch (sk2) file and saving it as a TIFF Bitmap file (*.tif) and opening the file in Microsoft Paint 5.1 to convert to JPEG (*.jpg). The 2D file was then placed in the pdf using the TouchUp Object Tool. The file was then saved as an Adobe pdf file (see Additional Note[b]).

Documents containing the embedded virtual 3D images are available in the RIT Digital Media Library[10] for free download for educational purposes. The technology has also been further utilized to create self-directed exercises in topics such as dipole moments and conformations of *n*-butane and cyclohexane.

■ VIEWING EMBEDDED VIRTUAL 3D IMAGES

The pdf files that contain embedded 3D images can be viewed using either Acrobat Reader version 10.0.3 or newer or Acrobat Professional version 10.0.3 or newer on either the Mac or Windows environments. For Mac users, these files cannot be viewed in Preview, which is the default pdf viewer on most Mac OS computers. Viewing these pdf files with either Safari or Firefox using the Adobe plug-in on the Mac can be spotty and inconsistent. The files should be created or downloaded and opened directly in either Acrobat Reader or Acrobat Professional.

■ STEREOCHEMISTRY LESSON

The classroom experience reported in this article centers around the use of a self-directed computer-based lesson embedded with virtual 3D images as a tool to assist in teaching the stereochemistry of organic compounds during a laboratory period. Stereochemistry of organic compounds is one of the more challenging topics in the organic chemistry sequence. Stereochemistry has traditionally been taught by first presenting the 2D structures to students in the classroom, then asking them to convert those 2D images into 3D models using handheld molecular-model kits. However, as modern technology plays a larger role in education, the addition of virtual 3D molecular images can supplement or replace the use of handheld models. The pilot study was conducted to gain initial feedback on whether the use of virtual 3D images in teaching stereochemistry should be pursued.

Table 1. Student Itinerary for the Stereochemistry Lab Session

1. Asked to read prelab (pre-lab pdf) before lab session
2. Worked through self-guided lab manual (lab manual pdf) with 2D structures and embedded virtual 3D images on the computer using molecular-model kits
3. Completed the lab exercise document (lab exercise pdf) on the computer. The students were given 2D drawings, molecular-model kits, and virtual 3D images to complete the exercise
4. Completed the lab quiz (lab quiz pdf) on the computer with only the use of 2D drawings
5. Completed a survey (survey pdf)

An organic chemistry class composed of mainly biochemistry and chemistry majors was given the instructions listed in Table 1 during their laboratory session in week 5 of the 30 week long organic chemistry sequence. The lab session was held in a personal computer classroom and each student used a computer to access the online lessons. The self-guided discovery documents were designed to engage the students in learning stereochemistry as a supplement to the lectures with hands-on work with stereochemical tools to develop their spatial ability skills and understanding of the stereochemistry of organic molecules. The students had the use of handheld molecular-model kits and pdf documents containing both the embedded virtual 3D images and the static 2D images. The students worked at their own pace and most required the majority of the 4-h lab period to complete all of the tasks. The self-guided lab manual required the most time and allowed the students to learn and correct their answers to questions. Once the lab manual was completed, the students were asked to complete the lab exercise consisting of 10 questions designed to test their stereochemistry knowledge. During the lab exercise, the students had access to the virtual 3D images in addition to the 2D drawings, and molecular models. After each

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Table 2. Survey Results[a]

Question	Strongly Agree (%)	Agree (%)	Disagree (%)	Strongly Disagree (%)
1. Were the virtual 3D images easy to interact with?	39	61	0	0
2. Do you think the virtual 3D images would be helpful in learning other topics in organic chemistry? In other courses?	39	61	0	0

Question	Added to the Lab	Detracted from the Lab	Both/Indifferent
4. Do you feel that the addition of the virtual 3D images added to or detracted from the effectiveness of the lab?	91	0	9

[a] See Additional Note[c]

question in the lab exercise, the students were asked to identify which stereochemical tool(s) they used to answer the question. Students were then asked to complete a 10-question stereochemistry lab quiz using only 2D drawings. At the end of the lab period, students were then asked to complete a short survey aimed at determining their feelings and thoughts about the virtual 3D images (see Additional Note[c]).

■ RESULTS

The results include the students' answers to a survey of which stereochemical tool they used in answering questions in the exercises (handheld molecular-model kits, virtual 3D images, both, or neither), an attitude survey at the end of the lab session, and instructors' observations.

Survey of Study Group: What Did the Students Think?

The students completed the survey at the end of their laboratory session and a summary of the questions pertaining to the virtual 3D images can be seen in Table 2 (see Additional Note[c]). The students seem in agreement that the virtual 3D images were easy to use. In answering question four, none of the students thought that the virtual 3D images detracted from the lab and 91% thought that the images added to the lab. The students demonstrated by their answers to survey questions two and four an acceptance of the virtual 3D images and an overall positive impression (Table 2).

Another point worth discussing is the ease of use of the virtual 3D images and the fatigue associated with extensive handheld molecular-model building. Question one of the survey demonstrates that the students are comfortable with the simplicity of using the virtual 3D images and during the lab sessions very few questions were a result of navigating the documents or using the images. During the 4-h lab, students did suffer from fatigue and several of them made comments to this effect in their surveys. Two survey comments are

> "...but 4 straight hours of packing stereochemistry into your brain is a bit much, ..."
>
> "I relied more on the 3D images just because it was so much work to put the hand kits together. Also, they were better in the sense that they were already loaded and could rotate them without worry about messing up."

Lab Exercise Results

During the stereochemistry exercise, after each question, the students were asked whether he or she used handheld molecular-model kits, virtual 3D images, both, or neither to answer the previous question (see Additional Note[c]). On the basis of their responses, the students used a stereochemical tool, either handheld molecular-model kits, virtual 3D images, or both, 37% of the time when completing the problems in the exercise. Of the times that the students used an additional tool, the virtual 3D images were used 74%, handheld molecular-model kits were used 20%, and both were used 6%. Therefore, when the students felt they needed the use of an additional stereochemical tool, the majority of the time they relied on the virtual 3D images.

Problems 3, 5, and 8 of the lab exercise can be most logically solved with higher spatial reasoning, which would be greatly assisted with handheld molecular models, or virtual 3D images, so intuitively one could assume the students would use the tools provided to obtain the correct answer (Table 3). In Table 3, the percentage of the students who got the question correct is listed in parentheses. For example, in problem three, 89% of those who used the virtual 3D images chose the correct answer, compared to 50% when students used only the handheld molecular kit and 23% when students used neither tool.

Instructor Observations

It is important to note some observations from the lecture and laboratory instructors. Two observations were made during the lab periods. First, the students were thoroughly engaged in the lab with the right amount of discussions between individuals. The computer seemed to captivate the students' attention. Therefore, having the self-directed documents available on the computer was largely responsible for engaging the students.[11] Second, the students complained of hand fatigue due to building molecules with the handheld molecular model kit. Later in the lab period, molecules built by the students were often incomplete.

■ CONCLUSIONS

Two-dimensional drawings of molecules will remain the general symbolic representation of molecular structures for the foreseeable future. One of the tasks as chemistry instructors is to equip students with the ability to mentally convert a 2D representation of a molecule into 3D and back to a 2D structure. Mastering this skill enables the students to relate structure to function and to envision potential interactions for that structure in 3D space.

The embedded virtual 3D images in the readily accessible portable document format (pdf) have the potential to make 3D molecular modeling accessible to anyone who has a computer and connection to the Internet. Instinctively, we believe that our students are growing more oriented toward learning in virtual online environments and we are committed to refining our materials and assessment tools to identify approaches and audiences that will receive the greatest benefit from this approach.

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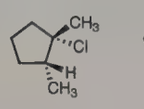
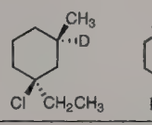
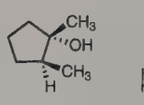
Table 3. Students' Responses to Three Laboratory Exercise Questions

Problem Number	Question: How are the following compounds related?	Number of Students[a]				
		Who Used the Handheld Molecular-Model Kit[a]	Who Used the Virtual 3D Images[a]	Who Used Both[a]	Who Used Neither[a]	Who Did Not Provide an Answer[a]
3	 ○ identical ◉ enantiomers ○ diastereomers	2 (50%)	9 (89%)	0 (n/a)	13 (23%)	0 (n/a)
5	 ◉ identical ○ enantiomers ○ diastereomers	0 (n/a)	13 (62%)	0 (n/a)	10 (50%)	1 (100%)
8	 ○ identical ○ enantiomers ◉ diastereomers	0 (n/a)	6 (100%)	0 (n/a)	17 (82%)	1 (100%)

[a] Number in parentheses is percent that got the question correct using the respective teaching tool.

On the basis of this experience, the students found the virtual 3D images useful and were successful in using them to solve problems in the exercises. The application of the embedded virtual 3D images in studying stereochemistry of organic molecules is intuitive and has the potential for increasing student learning and interest in organic chemistry. Initially, the focus has been on stereochemical understanding, but the use of embedded virtual 3D images could easily be extended to other structural aspects of chemistry.

■ FUTURE PLANS

Further development of the 3D materials can add depth to any online courses or online course work for general, organic, and biochemistry courses, but should not be limited to those disciplines. Handheld models are extremely useful in research, one's own learning, and in teaching, but the virtual 3D images are already prepared for the students and are not as cumbersome or limited by the quantity of atoms as are handheld molecular model kits. On the basis of the positive feedback from the students, the stereochemistry lab has been added to the curriculum and development of other self-directed learning exercises (e.g., virtual 3D images of molecules such as cyclohexane in higher energy conformations) using the virtual 3D images to enhance visio−spatial learning in the organic chemistry and biochemistry courses has begun.

■ ASSOCIATED CONTENT

Supporting Information

Copies of the detailed instructions for creating virtual 3D molecular images in a portable document format; prelab, lab manual, lab exercise, lab quiz, and survey form. This material is available via the Internet at http://pubs.acs.org.

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■ ADDITIONAL NOTE

[a] Detailed instructions with screen shots have been included in the Supporting Information.

[b] The 2D structures (step 1) could also be extracted from structure images in NCBI's PubChem Project[12] in a number of formats (InCHI, IUPAC International Chemical Identifier, and SMILES, Simplified Molecular Input Line Entry Specification) that Chemsketch can use to generate the desired structure. Using the "Clean Structure" Tool is often useful for optimizing bond angles and lengths.

[c] A copy of the document has been included in the Supporting Information.

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Frontiers of Crystallography: A Project-Based Research-Led Learning Exercise

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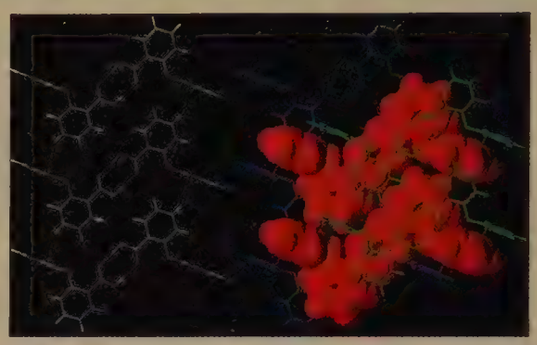
Supporting Information

ABSTRACT: A highly interactive research-led learning session for chemistry undergraduates is described, which aims to lead students to an awareness of the applications of crystallography technique through a mentored hands-on crystal structure solution and refinement session. The research-based environment is inherent throughout the 4.5 h program and is emphasized by several features in the learning experience.



KEYWORDS: Upper-Division Undergraduate, Analytical Chemistry, Curriculum, Problem Solving/Decision Making, Crystals/Crystallography, Hydrogen Bonding, Molecular Properties/Structure, Undergraduate Research, X-Ray Crystallography

Crystallography is one of the core techniques in materials characterization and forms a part of most undergraduate chemistry degrees. It has relevance to many industrial areas, in particular, the pharmaceutical industry where full identification of the precise molecular configuration in the crystal structure is vital as the molecular configuration can have significant effects on the properties of the material of interest. In addition, crystallographic screening of solid forms constitutes a significant part of the premanufacturing process, allowing phase purity to be established. Other relevant areas in which crystallography is widely used include the food, pigments and dyes, and agrochemical industries. It forms a key investigative technique in any area where the solid state is important and is unrivalled in the level of information available in terms of defining chemical connectivity, molecular geometry and conformation, including intermolecular interactions. Diffraction techniques can be used as a characterization tool for synthetic chemists determining the precise chemical arrangement of the atoms in the materials that they have made, but also as a tool toward understanding the physical properties of materials in the solid state where the weaker intermolecular interactions can have a significant effect on a range of physical properties such as solubility, melting point, and mechanical strength. This has led to the development of high profile areas such as crystal engineering. The theory of diffraction, however, can often leave students mystified and undergraduate students can consequently lose the relevance of the material that they are studying. A more hands-on approach to such problems can help to demystify these areas.

A "Frontiers of Crystallography" exercise has been introduced for upper-level undergraduate students in chemistry. This exercise is part of a series of themed "frontiers" afternoons comprising a supplementary course for undergraduate chemistry majors and is not assessed as part of the course-work.[1] A vital component of the course is its relaxed, informal environment, which is encouraged by the Friday afternoon timing, room layout, attitude of the tutors, and the nonconventional course content. However, this exercise could also be employed in a physical or analytical chemistry laboratory course over two periods.

GOALS

The aims of the exercise are not only to increase the understanding of crystallography by the undergraduate students, but also to provide the students with an opportunity to participate in the research process, undertaking an original research project over the course of two afternoon sessions, ideally resulting in the publication of this work in the form of a crystal structure report to a peer-reviewed journal. The learning process also allows the students to interact in an informal way with an active research group ranging from the professor in charge, to postdoctoral researchers (PDRAs) and Ph.D. students.

EXERCISE STRUCTURE

The exercise requires 4.5−5 h, over two sessions, deliberately set 2−3 weeks apart (to allow for sample preparation), with a typical attendance of 20−30 students. Prior to the Frontiers of Crystallography exercise, the students should have had classroom exposure to the concepts of diffraction, structure solution and refinement, and crystallographic symmetry and space groups.[2]

Session 1

The first session, 1.5 h, has the broad aim of providing an introduction to crystallography and structural chemistry, relying on the students' classroom knowledge of diffraction.[3]

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The introduction in session I discusses the importance of structural chemistry in one or more key science areas (for example hydrogen bonding or polymorphism). This allows not only a full contextual development of the course material, but also helps emphasize from the start the very strong research-driver for the course. Moreover, the specific materials (or range of materials) chosen for study on the research component of the course are introduced in this context. Specifically, it is made clear that the materials to be studied fit into ongoing research programs (indeed they often form part of the project of a PDRA or Ph.D. student who will be involved in the hands-on research sessions).

This relationship of the materials to be studied to local research interests is appropriate for a university or college with a strong research program and related crystallography facilities. However, this does not preclude undergraduate-only institutions from undertaking a similar exercise. For institutions without a strong research presence in crystallography, the Frontiers of Crystallography concept can still be a possibility. Various types of assistance are available to help establish such a course, including availability of the main organic crystal structure database, the charitably operated Cambridge Structural Database (CSD).[4] The major national crystallographic associations, notably the American Crystallographic Association (ACA) and the British Crystallographic Association (BCA), have dedicated Educational Committees as a core part of their remit and operation, providing a professional central contact point from which to secure advice and relevant contacts. In terms of target materials, the identification of likely hydrogen-bonding patterns is an interesting exercise in its own right, covering core aspects of structural chemistry and could easily be carried out as an assignment prior to the Frontiers workshop, supported by access to structural information from the CSD. There are also many crystallographic groups who would be happy to share some of their more "routine" materials for potential inclusion in such courses. In many cases this assistance could also extend to providing the data for the exercise.

The research emphasis is strengthened in this part of the introduction by holding a discussion session, sometimes modeled along the lines of a "Café Scientifique" open Q&A session.[5]

The more practical aspects of session 1 include a tour of the X-ray facilities used by structural chemistry researchers in the School of Chemistry, and on which the session 2 "hands-on" components are carried out. These tours are led by the course organizers or by PDRAs or senior research students from their groups. The tour emphasizes the research-level instruments to be used in the Frontiers exercise, and these will typically include a Bruker AXS Apex-II CCD-based diffractometer and a Rigaku R-axis/RAPID image plate diffractometer. Both instruments are state-of-the-art and utilized full-time on research projects in the School of Chemistry.

The tour of facilities is augmented by observation of typical sample preparation procedures used to grow the crystals utilized in the Frontiers sessions. It is usually necessary to set up these crystallizations in advance, as material preparation can take several weeks to produce crystals of sufficient size and quality for the experiments to be carried out. Future plans include the possibility of making this session hands-on, if session 1 were to be extended. Feedback from students has indicated enthusiasm for this extension of the "hands-on" experience in sample preparation, which would have to be scheduled significantly in advance of the structure solution workshop. This is accessible in any wet chemistry laboratory as sample preparation, the growth of small

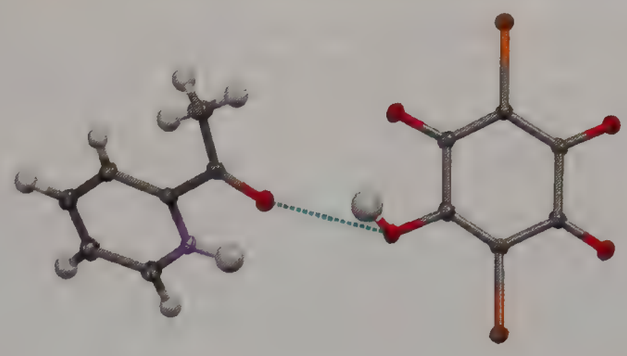

Figure 1. The hydrogen-bonded molecular complex that is the building block of a structure solved during a Frontiers of Crystallography exercise; 2-acetylpyridinium bromanilate.[6]

single crystals of organic materials, is usually based on simple slow solvent evaporation techniques. If such sample preparation is successful, then for those without access to "in-house" crystallography facilities, links with external crystallography groups or organizations could offer the opportunity of external data collection on samples for use in a Frontiers session.

Throughout session 1 it is emphasized that the Frontiers exercise includes a real research component. As such, the possibility of "failure" is emphasized: good quality crystals may not be grown, there may be problems in obtaining data, and there may be problems in solving and refining the crystal structure. There is no attempt made to "pre-screen" the information provided to the students; at every stage the students see the data as the researcher would see it. It is stressed that those involved in tutoring also do not know the answer; though they have an idea of what might emerge, sometimes it does not work out and sometimes something completely different is found. For example, typically a molecular complex is chosen (hopefully produced by co-crystallization of two distinct components that might hydrogen bond to each other in the solid state). Sometimes this works and is regarded as a triumph of "crystal engineering" (an example of a structure solved during a Frontiers exercise is shown in Figure 1), whereas sometimes a complex is obtained with a totally different intermolecular bonding motif. Sometimes crystals of the individual components are obtained, sometimes new forms (polymorphs) of the individual components are produced, sometimes more than one form of the desired complex is produced, for example polymorphs, or different stoichiometric ratios, and sometimes crystals of such poor quality are obtained that they cannot be used. All of these are genuine exposures to the real research experience!

Session 1 Outcomes

The anticipated outcomes from session 1 include (i) an awareness of why structural chemists do what they do and how they do it, which builds on a basic knowledge of crystallography and is applied in a research-led context; (ii) an introduction to research-level experimental techniques; (iii) initial exposure to the advanced equipment used for X-ray crystallography; and (iv) an increased interest in further study.[7] A very high return rate for session 2 is anticipated and usually delivered.

Session 2

Session 2, 3 h, is the practical hands-on data collection, structure solution, and refinement component of the Frontiers of Crystallography exercise. It is based on determining an unknown crystal structure, using samples prepared from crystallization

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experiments observed by the students in session 1. A team of 6−7 delivers the session, comprising academic faculty, PDRAs, and Ph.D. students from research groups related to the area of study; members of the team mentor individual small groups of undergraduate students, leading them through the process.

The students typically work in groups of 4−5, analyzing the structure of a series of crystals for which full data sets have been collected by members of the research team prior to the session. In early workshops, a single structure was studied by all groups, but as a result of feedback from various sources (including participants), this has been modified to ensure, as far as possible, that each group has a data set from a distinct material. The full data sets typically take 6−12 h to collect and so are collected in preparation for the structure solution and refinement components undertaken in the practical session. If more than one target material appears to have crystallized, where possible, data sets are collected from each of these. In that case, it may be that one of the research investigations produces better results than another, depending on the system studied; again this is part of the research-driven learning experience for the students. Where single crystals are unable to be grown, powder X-ray diffraction is used to give characteristic fingerprint patterns of the materials obtained and these can be compared with one another and with patterns from the starting materials to assess whether a previously unknown material has been obtained.

The session is split into two parts: (i) data collection and (ii) structure solution and refinement. In the first part, the students are taken through the process of how to assess the quality of candidate crystals for the diffraction experiment and how to select a suitable single crystal on which a full data collection could be attempted. The hands-on component of this makes use of optical and polarizing microscopes. Once the students have selected a crystal (of typical linear dimension 0.2−0.4 mm), it is mounted onto a fine glass fiber attached to a goniometer by manipulating the crystal, again under a microscope. This is then transferred to an X-ray diffractometer ready for data collection. A unit cell data collection is then carried out on the crystal, which involves collecting a series of frames of diffraction spots on the area detector; this typically takes around 30 min. The quality of the crystal can again be assessed by the quality of the diffraction spots observed; often the students discover that the crystal they have initially selected does not show good diffraction characteristics. Once good patterns are obtained, the computer software is then used to determine the unit cell parameters and these are compared with the known parameters obtained from the full data collection to identify whether this crystal is the same material. The students are then shown the steps involved in setting up a full data collection but without collecting data at this stage.

The data analysis involves using the data already collected, to solve and refine the unknown crystal structure. This is carried out in small groups, each around a PC or a laptop with research-level software installed. To expedite the process in the 1.5−2 "live" hours available for this procedure, the structure solution and refinement is based on a template (see the Supporting Information). Each group is mentored by a Ph.D. student, with the course leaders adopting a purely advisory role, including asking relevant questions to ensure a degree of understanding of the procedures.

It is stressed again throughout this process that no one knows the "correct" structure, so it is a full research-based experience. This is frequently manifested as different groups often take different paths though the process, depending on the practice of the facilitator. It is also possible that some groups end up with a "better" solution than the others, even if tackling the same structure, which leads to discussion of the reasons why and of what could be done to ensure that both solutions represent the true situation (usually meaning that one or more groups are encouraged to revisit their conclusion).

Once the structure has been successfully solved and refined, the students are led through the process of analyzing the structure, drawing out information that may be used in a scientific paper. This includes looking at the molecular conformation and also the three-dimensional crystal packing including any interactions such as hydrogen bonding. The template for analyzing and recording findings is shown in the Supporting Information. The students also make use of the Cambridge Structural Database[4] to ensure that the structure that they have found is new and to see if there are any similar structures already reported in the literature. The findings of all the groups are pulled together at the end to help write the comment part of the paper or papers that are the additional potential outcome of the process. Additional assignments are set to augment this information.

Session 2 Outcomes

The anticipated outcomes for session 2 include (i) use of advanced research-level instruments (diffractometers) and software; (ii) solution and refinement of a new, previously unknown, crystal structure; (iii) an exercise in making relevant observations on the structure that makes use of the information on related structures in the Cambridge Structural Database; (iv) interactions in a group and with a graduate student mentor, and (v) an awareness that in original research there is no "right" answer. This is emphasized by the different paths used for solution and refinement, and in some cases different final models that may or may not be equally valid. Sometimes the structures determined are not of great quality, possibly due to poor crystal quality and so forth; this is accepted as the outcome of the course—another example of research reality.

If all works out, a short paper on any suitably high-quality structures determined will be written up for one of the crystallographic structure reporting journals such as *Acta Crystallographica Section E* or *Zeitschrift fur Kristallografie: New Structures*,[6,8] with the potential inclusion as coauthors of those in the class who worked on the publishable new structure. In assessing initial outcomes, and with feedback from participants, the criteria for inclusion as an author on any resulting paper have been discussed, together with general discussions on publication. For similar exercises adopted elsewhere, this discussion is recommended as it covers a range of important educational and ethical issues. In the case of Frontiers of Crystallography, criteria based on additional research-related assignments that must be undertaken to "qualify" for coauthorship have evolved; these are focused on elements that will be included as background to the paper or to its discussion. Although these are only assessed informally, they are an important part of the process and ensure that genuine contributions are offered by all coauthors. Early engagement with editors is important, but it is clear that the "publication" outcome is likely to be most relevant for schools with crystallography research available or those who have been able to make a link with professional crystallography input through the routes outlined above. In general, editors are pleased to receive these papers and fully appreciate the reason for the often-extensive author list.

In some cases, the structures determined are of insufficient quality to warrant publication; again a reflection of the genuine

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research experience! In cases such as this, the research group will follow-up with efforts to obtain improved crystals and data sets and the undergraduate participants kept informed of progress on these.

■ SUMMARY

The Frontiers of Crystallography exercise is a research-led form of teaching that results in the exposure of students, in a relatively short and informal period, to a range of vital principles of the nature of research. These include awareness of the research process; experience of practical crystallography in a research environment; familiarity with modern structural chemistry techniques and research drivers; an ability to adapt to a problem with a genuinely unknown solution and "no right answer"; an experience of recording results and conclusions in a manner consistent with publication of original research; and hopefully, a published short paper.

These research-led learning outcomes are generic and do not apply solely to the area of research explored, and this is emphasized to the students. It is rewarding that many of the students who participate in the exercise take up Ph.D. positions in the school and elsewhere, including a fair proportion in the research groups of the course organizers. It may be that participation in the Frontiers of Crystallography exercise helps fuel the enthusiasm of the participants for a research career.[9]

An important aspect of an exercise such as this, and one that is easily neglected, is the chance for the class to meet the "true persona" of the academic staff. Rather than someone standing at the front of the class, lecturing on what may be rather dry subject matter, in session 1 the students get to see the academic in their natural environment, talking about research in a relaxed and interactive way. For the majority of students, this is their first exposure to a research environment, and it can come as a surprise to find the staff so passionate about their work. The students are responsive to this environment, and after the usual ice breaking, they welcome the opportunity to "just ask anything" in this general area. This is particularly emphasized in the mini-Café Scientifique sessions. It is also illuminating for the students to ask questions to which the academics simply do not know the answer, stimulating further discussion.

■ ASSOCIATED CONTENT

Supporting Information

An example worksheet that is used as prompts to guide the student participants though the practical aspects of the Frontiers sessions. This material is available via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We thank all participants in these Frontiers exercises over the last five years at the University of Glasgow: around 120 undergraduate students who have participated with enthusiasm and 12 mentors whose expertise and engagement have helped make these sessions a success.

■ REFERENCES

(1) At the University of Glasgow in the United Kingdom, a Frontiers of Crystallography exercise has been introduced that is delivered to level-3 MSci undergraduate students in chemistry (the MSci undergraduate curriculum at Glasgow is delivered over five years, with an industrial or international placement as year 4). The exercise is part of a series of themed "Frontiers" afternoons comprising a supplementary course for the students, and although formally compulsory, is not assessed as part of the course-work. The students attending the course are generally on one of the two major degree course streams (chemistry or chemistry with medicinal chemistry), or one of the three minor streams (chemistry with forensic studies, chemical physics, or chemistry with mathematics) within the chemistry course.

(2) The curriculum at the University of Glasgow includes a level-3 course in diffraction, which introduces crystallographic symmetry and space groups. This is taken by all MSci chemists including those taking the medicinal chemistry major. Equipped with this, and an associated laboratory-based exercise on structure solution and refinement, the students are sufficiently prepared to contribute to the interactive Frontiers exercise.

(3) At the University of Glasgow the diffraction course is offered in parallel with the Frontiers sessions.

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(9) Frontiers of Crystallography exercise has strong spin-offs into course assessment, in that several essay choices usually emerge from within the cohort attending the Frontiers course, supervised by one of the academic faculty involved.

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Learning about Intermolecular Interactions from the Cambridge Structural Database

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ABSTRACT: A clear understanding and appreciation of noncovalent interactions, especially hydrogen bonding, are vitally important to students of chemistry and the life sciences, including biochemistry, molecular biology, pharmacology, and medicine. The opportunities afforded by the IsoStar knowledge base of intermolecular interactions to enhance the educational experience of students are summarized. Derived from experimentally determined crystal structures in the Cambridge Structural Database and Protein Data Bank, IsoStar contains geometrical and spatial information on nearly 30,000 different types of interactions in small molecules and proteins.

KEYWORDS: First-Year Undergraduate/General, Upper-Division Undergraduate, Biochemistry, Chemoinformatics, Organic Chemistry, Inquiry-Based/Discovery Learning, Internet/Web-Based Learning, Hydrogen Bonding, Noncovalent Interactions, X-ray Crystallography

A clear understanding and appreciation of noncovalent interactions, especially hydrogen bonding, are vitally important to students of chemistry and the life sciences, including biochemistry, molecular biology, pharmacology, and medicine. In chemistry, noncovalent interactions of all types, whether mediated by hydrogen or not, are fundamental to the burgeoning field of supramolecular chemistry.[1] In the life sciences, hydrogen bonds are responsible for the structural organization of DNA, RNA, and the higher-order structure of proteins and are crucial in protein−ligand interactions that are fundamental to the activity of pharmaceutical or agrochemical ingredients (APIs or AAIs).[2]

Several monographs provide excellent historical background on hydrogen bonding (e.g., refs 3, 4). The term "weak bond" was first described by Latimer and Rodebush in 1920[5] and the more specific "hydrogen bond" terminology was first described by Pauling in 1935.[6] In both cases, the terms were invoked to explain chemical observations. In his famous 1939 monograph,[7] Pauling brings the hydrogen bond into mainstream chemistry, and defines it as an electrostatic interaction of the form $D-H^{\delta+}\cdots A^{\delta-}$ involving a donor H atom (D−H) and a suitable electronegative acceptor atom (A). Other donor−acceptor interactions are, of course, possible, and a wide range of interactions not mediated by hydrogen were discussed in a seminal review by Bent in 1968[8] and more recently by Diederich and co-workers.[9]

While hydrogen bonding and other D···A interactions can be studied using a variety of techniques, the vast majority of our current knowledge comes from X-ray and neutron crystallography (see e.g., refs 4, 5). A crystal structure comprises an infinite array of molecules and/or ions having an extended structure that is stabilized by a wide range of noncovalent interactions, but dominated by hydrogen bonds wherever these can form. Because atomic coordinates in small-molecule crystal structures are determined experimentally at high resolution, the crystallographic technique provides direct and accurate observations of the metrical and spatial characteristics of individual interactions. Although many examples taken together can provide indirect evidence of relative interaction energies and competition effects in structures containing multiple donors and acceptors, it is common[10] to combine analyses of crystal structure information with energy calculations at various levels of theory.

In a series of recent articles,[11−15] we have described the Cambridge Structural Database (CSD)[16] of small-molecule organic and metal−organic crystal structures and discussed a wide variety of teaching modules based on the three-dimensional (3D) structural information in the CSD. In particular, we showed how the complete CSD system can be used to locate examples of N−H···O hydrogen bonds in amides and examine the geometrical and spatial characteristics of these bonds.[14] Such studies are typical of hydrogen-bond research using crystal structure information but they require a clear understanding of precisely which systems to examine, as well as knowledge of the CSD system software[13] required to carry out the database analysis.

To bring alive the world of intermolecular interactions, the Cambridge Crystallographic Data Centre (CCDC) has also developed a knowledge base, IsoStar.[17] This Web application provides thousands of interactive 3D scatterplots that show the probability of occurrence and spatial characteristics of interactions between pairs of chemical functional groups. Accessed using just a few intuitive button clicks, these scatterplots allow students to readily investigate intermolecular interactions without the need to construct complex search queries or carry out detailed data analyses. Scatterplots and numerical data are derived from the CSD and from the Protein

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Table 1. Summary of Central Groups Available for Selection in the IsoStar Knowledge Dase of Intermolecular Interactions

Group Class (N_g)[a]	Examples
Terminal Groups[b]	
C, H only (7)	methyl, ethyl, ethynyl, methylene, i-propyl, vinyl, t-butyl
N, C, H only (20)	amino, azido, imino, cyano, diazonium, guanidinio
O, C, H only (13)	formyl, acetyl, acetoxy, carboxylic acid, hydroxy, methoxy
N, O, C, H only (11)	formamido, carbamoyl, hydroxyimino, nitro, nitroso, ureido
Si-containing (1)	trimethylsilyl
P-containing (4)	phosphato, phosphonato
S-containing (13)	thio, acetylthio, thiocyanato, sulfato, methylsulfonyl
Halogen containing (11)	aliphatic and aromatic C-X, dichloromethyl, trifluoromethyl
Acyclic Links	
C, H only (9)	ethane-, ethene- and ethyne-1,2-diyls, methylene, tertiary C−H
N, C, H only (10)	amine, azo, hydrazone, iminomethyl, tertiary ammonium
O, C, H only (15)	ester, ether, ketone, carbonate
N, O, C, H only (11)	amide, azoxy, carbamate, hydroxyimino, urea
P-containing (4)	C−O−P, P−O−P, phosphinate, phosphonate
S-containing (18)	disulfide, dithioester, sulfonamide, sulfone, thioamide, thione
Ring Systems	
Phenyl (30)	-C_6H_5 plus 29 common substituted phenyl systems
C, H only (13)	cyclo-(propyl,butyl,hexyl), indan, naphthalene, fused benzenes
N, C, H only (37)	imidazole, indole, aromatic N systems, pyrazole, pyrrole
O, C, H only (15)	epoxide, furan, lactones, benzoquinone, coumarin, aromatic-O
N, O, C, H only (26)	oxazole, lactams, barbituric acid, hydantoin, prolyl
S-containing (9)	isothiazole, penicillin, aromatic-S, thizole, thiophene
Nucleic acid bases (7)	adenine, cytosine, guanine, thymine, uracil
Solvates, etc.	
Inorganic (1)	water
Organic (14)	acetone, benzene, methanol, toluene, pyridine, chloroform
Protein Related	
Terminal groups (10)	carboxylic acid, hydroxy, methyl, methylthio, carbamoyl
Links (2)	peptide, disulfide
Ring systems (14)	phenyl, 4-hydroxyphenyl, prolyl, imidazole, indol-3-yl

[a] The number of central groups in each class, N_g, is given in parentheses. The total number of central groups covered in IsoStar is 319. [b] To aid selection, central groups are classified according to their elemental composition and broad chemical functionality (terminal, acyclic links, rings, etc.).

Data Bank (PDB),[18] and interaction energies, computed using intermolecular perturbation theory (IMPT),[19] are also included for many important systems. Originally designed to survey likely protein−ligand binding mechanisms in drug discovery projects, IsoStar also has some fundamental educational applications that will be discussed and illustrated in the present article.

■ THE ISOSTAR KNOWLEDGE BASE

Overview

The core of IsoStar is a set of 3D scatterplots. Each scatterplot has been calculated by searching the CSD or PDB for nonbonded interactions between a pair of functional groups A and B. The A···B contacts are transformed so that the A groups are least-squares superimposed. The resulting scatterplot shows the experimentally observed distribution of B (the contact group) around A (the central group). The range of central groups and contact groups available in IsoStar is summarized in Tables 1 and 2. Scatterplots give information about the frequencies and directionalities of intermolecular contacts. The CSD-based distribution of O−H donor contact groups around an aliphatic ketone central group, a strong H−bond acceptor, is shown in Figure 1. The basic 3D IsoStar scatterplot, which can be rotated and manipulated within the IsoStar application, is shown in Figure 1A. Figure 1A shows the distribution within an O···O distance limit of the sum of van der Waals radii (ΣvdW) plus 0.5 Å, which is the standard default for all initial IsoStar plots. However, the system will also display an overview of the numerical data, and the histogram of O···O distances relative to ΣvdW is shown in Figure 1B. This histogram shows a strong peak corresponding to true H-bonds at distances, $d <$ ΣvdW − 0.5 Å, and the scatterplot can then be restricted to display only these H-bonded O−H···O contacts as in Figure 1C.

Showing the contact groups as wireframe bonds (the default IsoStar representation as in Figure 1A) does not, however, reveal preferred contact geometries in a visually accessible way, so IsoStar also provides a facility to convert scatterplots into contoured density surfaces. The surfaces are calculated by dividing the space around the central group into a regular grid and counting the observed number of contact-group atoms in each grid volume. This produces an empirical estimate of the density of contact-group atoms at each of a regular matrix of

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Table 2. Summary of the Contact Groups Available for Selection in the IsoStar Knowledge Base of Intermolecular Interactions

Contact Group Type[a]	Examples
General groups	i.e., any C, N, O, S, or H atom
Any polar hydrogen	X−H, where X is N, O, or S
Hydrophobic groups	e.g., methyl, phenyl
Specific N−H hydrogen-bond donor groups	e.g., amide NH, ammonium NH, etc.
Specific O−H groups	e.g., alcohol OH, water, etc.
Other groups containing N and/or O	principally H-bond acceptor groups such as cyano, carbonyl-O, nitro
Various types of sulfur containg groups	e.g., thioether, thiocarbonyl
Groups containing halogen atoms	e.g., C−F, chloride
Groups found in amino acids	e.g., imidazole, guanidinium

[a] The total number of contact groups available is 48.

grid points around the central group. The densities are then contoured at user-specified levels and displayed as surfaces. The contoured plot in Figure 1D highlights the regions in space where the O−H oxygen atoms accumulate. Here, the contour colors have been chosen so that blue and yellow denote regions preferred by the O atoms and red denotes the most preferred region. In a similar way, a surface could be produced showing where the O−H hydrogen atoms tend to be. A clear preference for O−H donors to approach the keto-O acceptor along the O lone pair directions is observed.

Interface

IsoStar is a client-server application and available scatterplots can be browsed and selected online using a standard Web browser. Once chosen, a particular scatterplot is automatically downloaded where it can be visualized and manipulated using a locally installed client application.

The IsoStar Home Page lists the central group types as shown in the left-hand column of Table 1. Selection of a central group type brings up a second selection pane that shows chemical diagrams of the specific groups within that type, that is, the groups exemplified in the right-hand column of Table 1. Selecting a specific central group generates a third selection pane that shows the range of contact group scatterplots that are available for that central group within the IsoStar system. Contact groups are organized by type, as shown in Table 2. The contact group selection pane is illustrated in Figure 2A for O−H contacts to a charged carboxylic acid central group, and the pane also shows the number of CSD or PDB observations that contribute to each scatterplot. For certain key interactions, a separate button (see Figure 2A) indicates that IsoStar also contains potential energy minima (interaction energies) derived using IMPT.[19] Clicking this button displays a table of the relevant energy minima from which a graphical representation can then be displayed (an example is shown later in Figure 4C).

Figure 2B shows the IsoStar client application that features two display windows allowing a pair of IsoStar distributions to be viewed, manipulated, and compared (if required) at any one time. Figure 2B illustrates contoured distributions of H-bonded O−H contact groups around charged carboxylate central groups in CSD entries (left) and PDB entries (right). Both plots show the expected lone-pair directionality and confirm the overall similarity of intermolecular interactions in high-resolution, small-molecule structures (CSD) and lower-resolution protein structures (PDB). Each plot can be activated independently, and buttons and sliders above the right-hand display control the contact distance ranges relative to ΣvdW to be displayed in the active plot and allow the data overview (Figure 1B) to be displayed in a separate pop-up window. Buttons positioned above the left-hand display control the contouring options to be applied to the active scatterplot. Right clicking in the active display window generates a pop-up submenu that controls display options, for example, the ability to change plot colors or to display atoms in a variety of styles. In the plots illustrated in this article, all central groups have been displayed in ball-and-stick mode for clarity. Additionally, because every single contact group within a scatterplot represents an interaction observed in a crystal structure, it is possible to click on an individual contact and hyperlink back to the specific CSD or PDB entry from which it was generated. This allows students to mine the IsoStar scatterplots and examine the underlying crystal structures in order to account for particular observations or apparent anomalies.

Information Content and Statistics

IsoStar contains information about intermolecular interactions derived from

- The CSD:[15] 22,161 scatterplots.
- The PDB:[17] 7,459 scatterplots derived from protein−ligand complexes having structures determined by X-ray diffraction at better than 2.5 Å resolution.
- Interaction energies (kJ mol^{-1}): 1,550 energy minima obtained using ab initio intermolecular perturbation theory (IMPT).[19]

The information content of IsoStar is updated annually and new central and contact groups are also added from time to time. Additionally, the IsoGen software module allows users to create their own data and scatterplots for interactions not yet covered by the IsoStar system.

Accessing IsoStar

A public IsoStar server is hosted at CCDC.[20] Access to scatterplots from this public server requires a licensed copy of the IsoStar client package. The IsoStar client package for supported Windows, MacOSX, and Linux platforms is distributed as part of the CSD system. The complete system comprising the database of over 500,000 crystal structures and associated search, visualization, and analysis software is supplied to individual institutions for a small cost-recovery fee, which is further reduced for non-Ph.D. awarding institutions. This fee is necessary because the CCDC is a not-for-profit organization that

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receives no public funding to carry out the work of collecting, evaluating, curating, and distributing database information and developing the associated software. All access enquiries should be directed to teaching@ccdc.cam.ac.uk.

■ TEACHING APPLICATIONS OF ISOSTAR

The following teaching examples are intended only to illustrate the range of educational opportunities afforded by the IsoStar knowledge base of intermolecular interactions and to demonstrate its potential to enhance the learning experience of students. No formal assessment of the learning efficacy of these specific examples has yet been carried out. Rather, our purpose here is to encourage educators to develop their own formal teaching exercises or open-ended discovery assignments based on these tools.

Electronegativity and Electropositivity

Electrostatic attractions are the key energetic component of favorable donor–acceptor interactions,[21] as already illustrated in the H-bond examples of Figures 1 and 2. This can be further illustrated for weaker interactions by examining IsoStar plots for the approach of both electronegative and electropositive contact groups to, for example, phenyl or ethynyl central groups. Figure 3 shows contoured IsoStar plots for the distribution of (A) carbonyl ($-C{=}O^{\delta-}$) groups and (B) any alkyl $-C-H^{\delta+}$ around a phenyl ($-C_6H_5$) central group. Figure 3A shows that the electronegative carbonyl-O atoms preferentially approach phenyl in the plane of the ring, that is, are oriented toward C(phenyl)$-H^{\delta+}$ forming weak C$-$H$\cdots$O hydrogen bonds.[4] Note that other electronegative species such as Cl^- also show the same preference for in-plane approach to phenyl rings, forming C$-$H$\cdots Cl^-$ hydrogen bonds. By contrast, Figure 3B shows that the weakly electropositive alkyl $C-H^{\delta+}$ prefer to approach the phenyl ring perpendicular to the ring plane; that is, they interact with the electronegative π-electron density above and below the ring forming C$-$H$\cdots\pi$ interactions.[22] Both of these effects can be seen in the interactions of $-$O$-$H contact groups with ethynyl central groups (Figure 3C). Here, there is a ring of O$-$H$\cdots\pi$ contacts surrounding the C≡C triple bond, and a group of H$-$O$\cdots$H$-$C≡C hydrogen bonds forming an O-surface directed toward the acidic electropositive ethynyl$-$H atom.

Strong Acceptors Compete for Donor-H Groups

Figures 1 and 2 show that carbonyl-O atoms in ketones and carboxylic acids are potent H-bond acceptors, and the same is true of ether-O atoms in, for example, aliphatic ethers $Csp^3-O-Csp^3$ as shown in Figure 4A. However, the IsoStar plot of H-bonded O$-$H contacts around aliphatic ester groups in Figure 4B shows that the majority of these bonds form to the terminal $-C{=}O$ rather than the etheric C$-$O$-$C. At first sight, this does not conform to expectations raised by Figure 1 and Figure 4A, so why should this be? IsoStar provides the answer in the form of the relative IMPT interaction energies,[19] as displayed in Figure 4C: in esters, the O$-$H$\cdots$O=C interaction is strong at -24.7 and -26.0 kJ mol^{-1} to the *E*- and *Z*-lone pairs, respectively, but the O$-$H$\cdots$O (ether) interaction energy is much weaker at only -15.0 kJ mol^{-1}. The energy difference of about 10 kJ mol^{-1} disfavors H-bond formation to the etheric oxygen, and the $-C{=}O$ is a clear winner in the competition for available donor-H atoms, as might be deduced from the resonance forms of the ester.

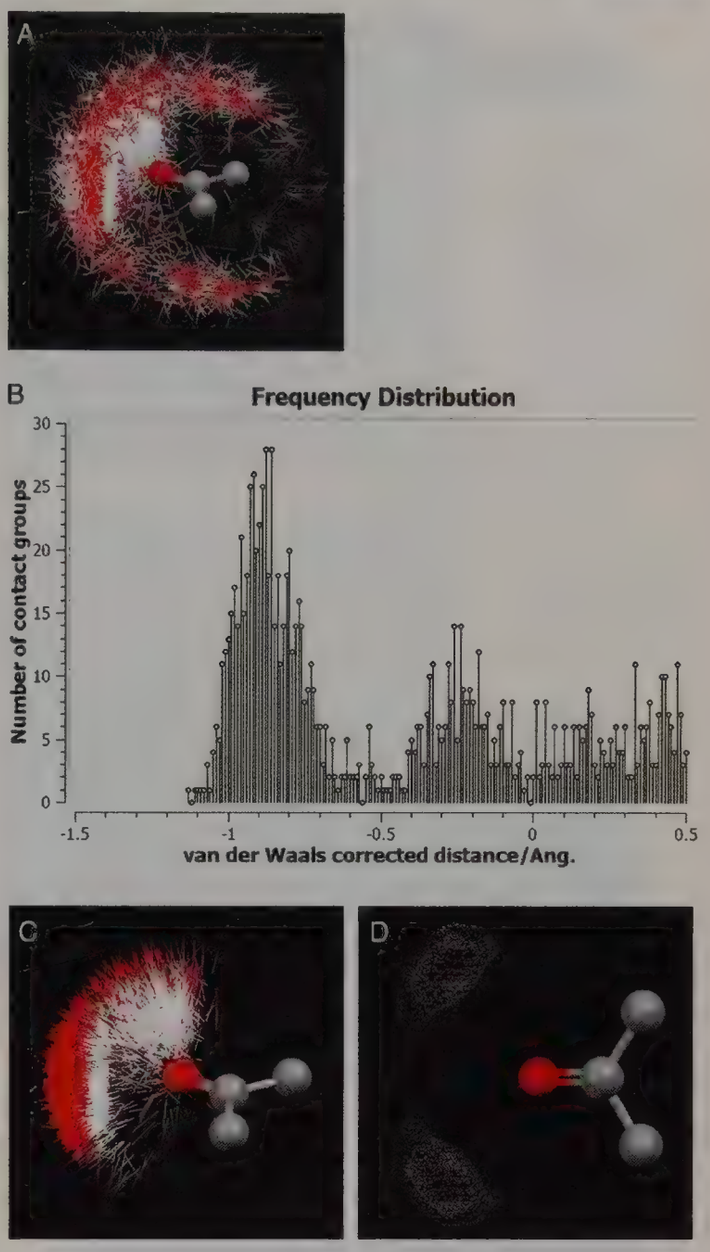

Figure 1. (A) Raw IsoStar scatterplot showing distribution of O$-$H donor contact groups around an aliphatic ketone central group, where red indicates the oxygen atoms, gray indicates the carbon atoms, and white indicates the hydrogen atoms. (B) Histogram of O$-$H$\cdots$O=C (aliphatic ketone) contacts, where H$\cdots$O distances are relative to the sum of van der Waals radii, ΣvdW (see text). (C) O$-$H$\cdots$O=C (aliphatic ketone) scatterplot truncated at contact distances, $d <$ ΣvdW $-$ 0.5 Å, and (D) O$-$H$\cdots$O=C (aliphatic ketone) contoured interaction-density surface plot of H-bonded contacts in (C); contour colors are described in the text.

Energy and resonance considerations also explain the clear preference for N$-$H and O$-$H contact groups to form H-bonds to the N atom of an isoxazole ring rather than the O atom, as shown in the IsoStar plot of Figure 4D. H-bond competition in isoxazoles formed part of an extensive study of competition effects[23,24] which showed that the IMPT interaction energy for N$-$H$\cdots$N (isoxazole) was -23.6 kJ mol^{-1}, whereas that for N$-$H$\cdots$O (isoxazole) was -15.7 kJ mol^{-1}, values that are now immediately available from the IsoStar system.

Dipole–Dipole Interactions Not Mediated by Hydrogen

The hydrogen bond is, of course, an interaction between two dipoles, for example, the $O^{\delta+}-H^{\delta+}\cdots O^{\delta-}{=}C^{\delta+}$ interaction

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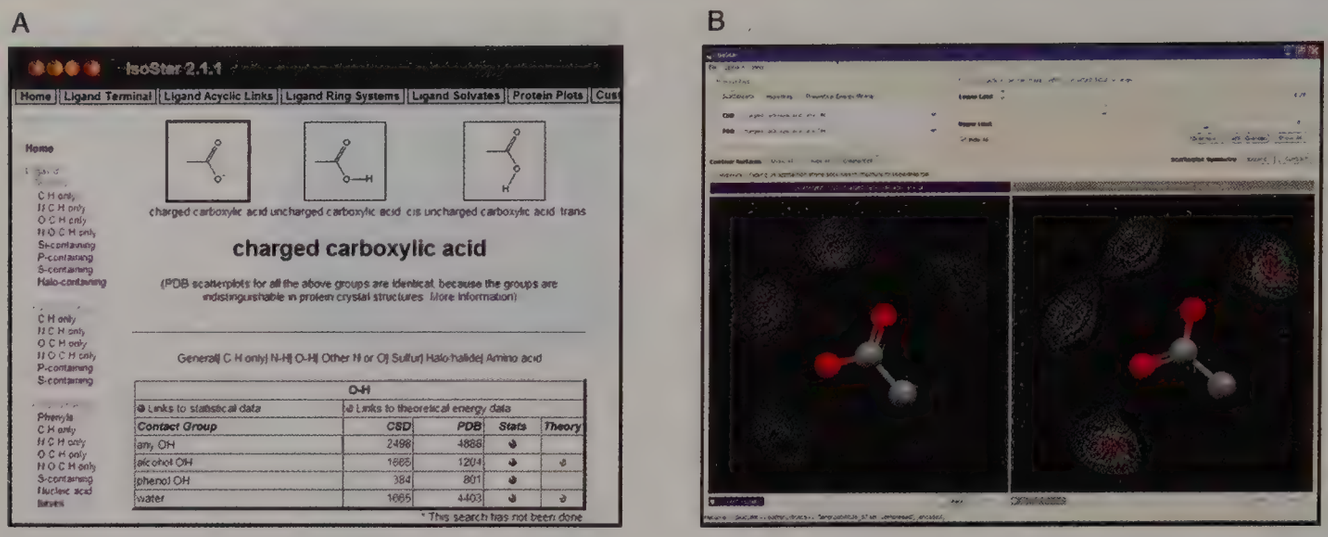

Figure 2. (A)The IsoStar interface showing the scatterplot selection pane for a charged carboxylic acid central group and the range of O−H contact group scatterplots that are available. (B) IsoStar client application displaying contoured plots of CSD data (left) and PDB data (right) for H-bonded O−H···O contacts to a charged carboxylic acid central group.

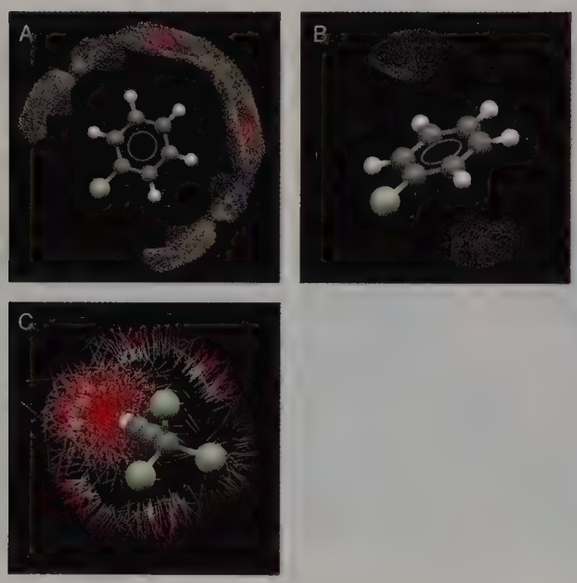

Figure 3. (A) Contoured interaction-density plot for the distribution of carbonyl ($-C{=}O^{\delta-}$) contact groups around a phenyl ($-C_6H_5$) central group. Red denotes regions most preferred by the carbonyl O atoms. (B) Contoured interaction-density plot for the distribution of any alkyl C−H contact group around a phenyl ($-C_6H_5$) central group. (C) Scatterplot showing −O−H contact groups around an ethynyl central group. Note the ring of O−H oriented contacts interacting with the ethynyl π-density and the H−O oriented contacts directed toward the acidic ethynyl−H atom.

that characterizes the IsoStar plots shown in Figures 1 and 2. However, the strongly dipolar nature of the C=O bond that makes its O terminus such a potent H-bond acceptor also makes this bond a candidate for other dipole−dipole interactions that are not mediated by hydrogen, as discussed elsewhere.[8,9] IsoStar is an ideal tool for studying these interactions, and Figure 5A shows an IsoStar plot of the mutual approach of carbonyl groups: the central group is an aliphatic carbonyl, the contact group is any carbonyl. In this plot, the interactions formed by the C of the contacting C=O have been contoured at two levels, colored in yellow and blue, whereas the O(C=O) interactions with the central group are contoured in red and green. It is clear that pairs of carbonyl groups prefer to associate in an antiparallel arrangement involving a pair of $C^{\delta+}\cdots O^{\delta-}$ electrostatic interactions. Students can examine these interactions in individual structures by using the IsoStar hyperlink feature to view CSD entries that contain some of the shortest C···O contacts. Figure 5B shows pairs of acetone solvent molecules forming antiparallel dimers in the CSD structure VAHVEF, with a C···O distance of 2.34 Å, well below the sum of van der Waals radii. These dipolar carbonyl−carbonyl interactions have been studied in detail, both in small molecules[25] and in proteins,[26] and the overall interaction energy for the antiparallel motif has been calculated[25] to be −23.5 kJ mol^{-1}, that is, the interaction is similar in energy to that of a medium strength hydrogen bond. Carbonyl−carbonyl interactions are responsible for the significantly higher boiling point of acetone over, for example, ethyl methyl ether, and in addition, these interactions have been shown to (i) have a substantial influence on β-strand, α-helix, and β-sheet secondary structure motifs in proteins,[26] and (ii) stabilize asparagine conformers that occupy partially allowed regions of the Ramachandran plot.[27]

HOMOs and LUMOs: Interactions at Divalent Sulfur

In 1977, Dunitz and co-workers[28] studied the interactions of divalent sulfur, X−S−Y, with electrophiles and nucleophiles. They used the molecular orbital model to explain that electrophiles should interact primarily with the highest occupied molecular orbital (HOMO), a sulfur lone-pair orbital that extends approximately perpendicular to the X−S−Y plane, whereas the nucleophiles should interact with the lowest unoccupied molecular orbital (LUMO), σ^*(S−X) or σ^*-(S−Y), lying along the extensions of the S−X or S−Y bonds. They note that in reaction pathway terms, these directions of approach represent the preferred directions of incipient electrophilic or nucleophilic attack on divalent sulfur. Figure 6 shows the IsoStar plots for a divalent sulfur having X=Y=aliphatic carbon as the central group, with (Figure 6A) any polar $N{-}H^{\delta+}$ or $O{-}H^{\delta+}$, serving as a model electrophile and (Figure 6B) any $C{=}O^{\delta-}$ serving as a model nucleophile. The plots show the clear preference for the electrophilic $H^{\delta+}$ to approach the HOMO roughly perpendicular to the X−S−Y plane (Figure 6A), with the nucleophilic $O^{\delta-}$ approaching X−S−Y along the LUMO along the S−X,Y bond extensions (Figure 6B).

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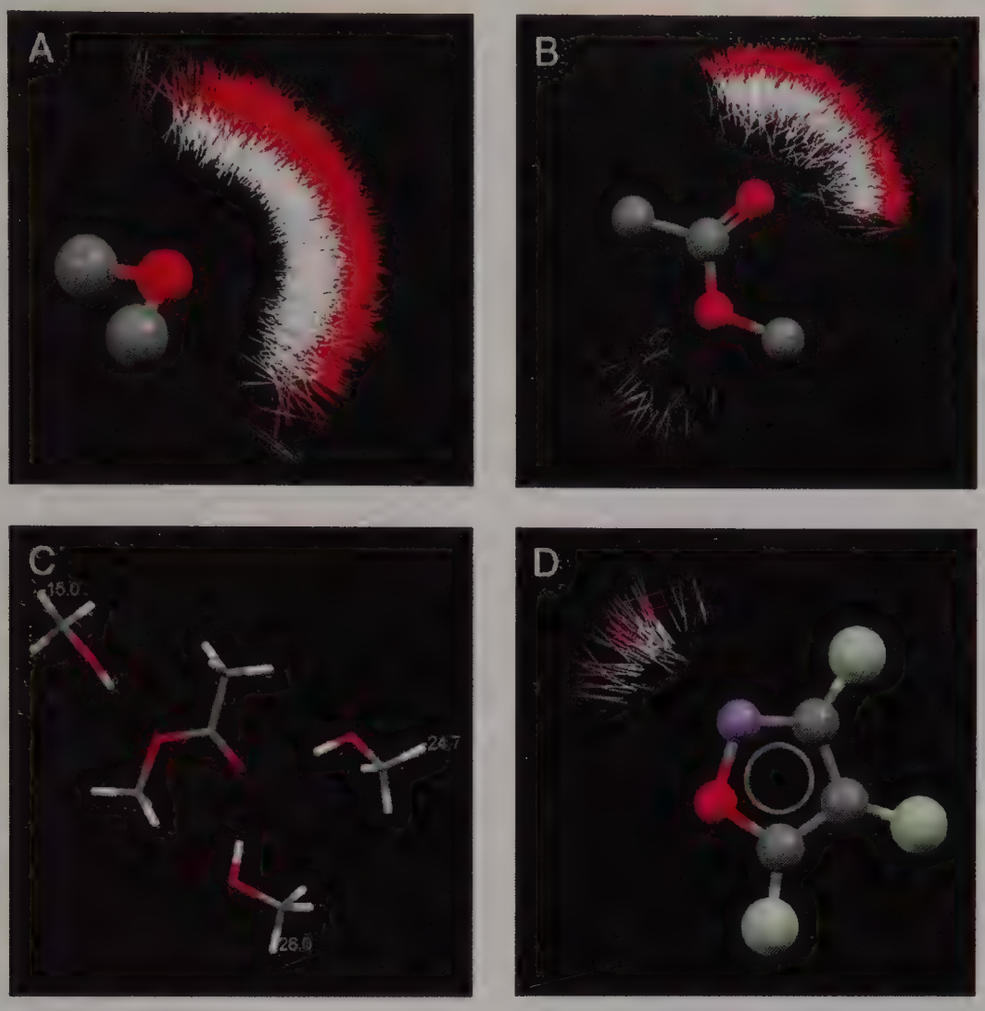

Figure 4. (A) Scatterplot of O—H···O (ether) H-bonds. (B) Scatterplot of O—H···O H-bonds in esters. (C) O—H···O interaction energies in esters (kJ mol ^{1}). (D) Scatterplot showing O—H and N—H H-bonded contacts to isoxazole rings, where the blue indicates the nitroge atom. The preference for N,O—H···N bonds is clear.

Figure 5. (A) Carbonyl—carbonyl interactions in IsoStar: approach of any carbonyl group (contact group) to an aliphatic carbonyl group (central group). The C-contacts are contoured in blue and yellow, and the O-contacts are contoured in red and green to reveal the preferred antiparallel dimer motif. (B) Antiparallel carbonyl···carbonyl motif in CSD structure VAHVEF viewed in IsoStar using the CSD hyperlink feature.

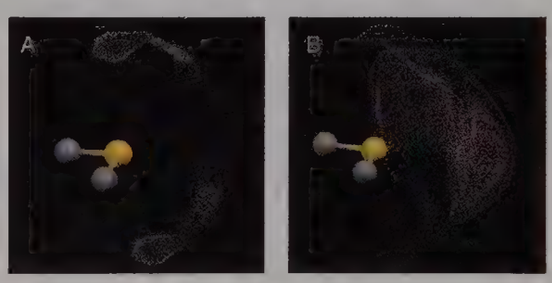

Figure 6. (A) Interactions of electrophiles (modeled using N—H and O—H contact groups) with a divalent sulfur central group (indicated by yellow). (B) Interactions of nucleophiles (modeled using a C═O contact group) with a divalent sulfur central group.

■ CONCLUSIONS

This article has summarized the opportunities afforded by the IsoStar knowledge base of intermolecular interactions to enhance the educational experience of students. IsoStar is a large system containing geometrical and spatial information on nearly 30,000 different types of interactions in small molecules and proteins. This crystal structure information is enhanced by the results of energy calculations on more than 1,500 key interactions. The simple and intuitive Web browser interface and interactive graphical presentation of results makes it an ideal tool for student exploration of supramolecular chemistry and structural biology. A short article such as this can only introduce the wealth of information available to educators and students, but the extensive facilities available in IsoStar for the exploration of hydrogen bonding and other important nonbonded interactions can only be fully appreciated by hands-on use. We would be grateful if IsoStar users could let us know about educational applications of the system, so that we may include these in the developing teaching section of the CCDC Web site.[29]

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ARTICLE

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Integrated Teaching of Structure-Based Drug Design and Biopharmaceutics: A Computer-Based Approach

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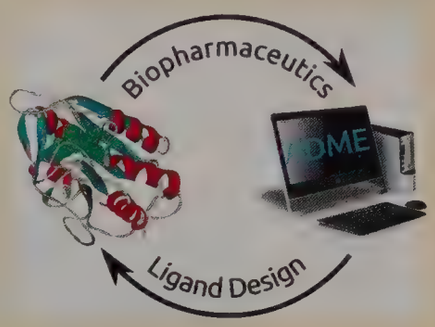
ABSTRACT: Rational drug design requires expertise in structural biology, medicinal chemistry, physiology, and related fields. In teaching structure-based drug design, it is important to develop an understanding of the need for early recognition of molecules with "drug-like" properties as a key component. That is, it is not merely sufficient to teach students how to design an effective inhibitor for a particular protein; instead, it is important to convey the need for simultaneous consideration of biopharmaceutical properties that will optimize the chances of the inhibitor becoming a drug. These are advanced concepts, but they can be addressed through computer-based methods. Here, an educational approach using a case study is described in which students "design" a potential drug through use of software, most of which is Web-based and freely available.



KEYWORDS: Graduate Education/Research, Upper-Division Undergraduate, Chemoinformatics, Interdisciplinary/Multidisciplinary, Computer-Based Learning, Internet/Web-Based Learning, Computational Chemistry, Drugs/Pharmaceuticals

Of the approximately 100,000 proteins in the human proteome, only about 500 are currently targeted by one of the approximately 40,000 drugs approved worldwide.[1] This suggests that there is considerable scope for identification of new targets and for the design and discovery of new drugs. On the other hand, the current average failure rate for drugs in clinical trials is 81%.[2] A lack of efficacy causes 30% of these failures, and concerns with toxicological and clinical safety account for another 30%.[3] Failure in the later stages of drug development is very expensive, particularly if a potential drug reaches phase II or III clinical trials before problems emerge. On average, companies are spending $27 million per year to advance drugs through clinical trials, at a total cost to market of over $1 billion.[4] Emergence of safety issues postapproval can have major costs of recall and legal fees, as demonstrated by the voluntary recall by Merck of Vioxx (rofecoxib) in 2004; this event has been estimated to have a cost of $9 billion in foregone profits and $5 billion in future litigation costs.[5]

These issues emphasize the importance of identification of molecules that are likely to reach the market at the early stage of drug discovery. In 1991, the industry observed a failure rate of 40% due to bioavailability and pharmacokinetic issues,[3] but incorporation of ADME (absorption, distribution, metabolism, and excretion) principles earlier in the drug development process to eliminate weak candidates has reduced failures for these reasons to 10%.[6] This approach has been facilitated by development of theoretical methods for prediction of "drug-like" properties of small molecules over the last 20 years. These have ranged from the simple, but effective and widely accepted, Lipinski "rule of five"[7] to sophisticated algorithms for prediction of ADME properties.[8,9] Input to these algorithms has been facilitated by use of text-based molecular representation through the powerful SMILES approach.[10]

Structure-based drug design against protein targets has been facilitated by the growing number of protein structures. There are currently 71,516 structures in the RCSB Protein Databank (March 1, 2011).[11] However, not all these proteins are targets for drug design. The number of "druggable" proteins (those that may be targets for drugs) was first addressed in 2002 by Hopkins and Groom,[12] with an upper estimate of 1500, and more recent reviews[13,14] suggest that this number remains valid. In 2006, Overington et al.[1] identified 1357 unique drugs that targeted 266 human proteins from examination of the FDA Orange Book[15] and the CDER Web site.[16] Thus, many druggable proteins have not been targeted. A goal of structure-based drug design[17] is to use structures of these proteins, or homology models derived from related proteins, to identify lead compounds computationally. The main method is molecular docking,[18,19] in which an attempt is made to locate molecules in a binding site using stereochemical and energetic considerations, with various simplifying assumptions depending on the required throughput.

This background presents a challenge for teaching of drug design at all educational levels. In 2006, Wild and Wiggins reviewed the development of educational initiatives and degree programs in chemoinformatics, in response to the growing availability of computer resources for storage and evaluation of molecular information.[20] Prior to this, Carvalho et al. had presented an interesting molecular modeling approach to active learning of structure—activity relationships and drug action, with a focus on enzyme mechanism and ligand fitting in an active site.[21] Cohen et al. have described strategies for teaching chemoinformatics and modeling in combination using commercial software

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(Molecular Conceptor), with a target audience of professional medicinal chemists.[22] At an earlier stage in the educational process, Gledhill et al. described a fascinating broad-based software approach that allows preuniversity students to perform hands-on drug design with Web-based distributed software.[23] Satyanarayanajois pointed out the importance of structural visualization for promoting understanding of basic principles in medicinal chemistry and biochemistry among professional pharmacy (Pharm.D.) students,[24] and Manallack et al. have described a detailed teaching strategy using molecular modeling, with a focus on GPCR targeting.[25]

Appreciation of the science of structure-based drug design requires familiarity with structural biology, thermodynamics, molecular association, stereochemistry, and computational methods; while appreciation of the biopharmaceutical properties of drugs (these are also commonly referred to as "ADME properties", but "biopharmaceutical properties" is used in this article because the focus is mainly on properties that influence absorption) requires an understanding of cell biology, physiology, preclinical formulation, and physical organic chemistry. The subject of structure-based drug design is only properly understood in the context of an integrated approach: this is a key in commercial drug design and it is also required in teaching of drug design. Here, a computational approach using Web-based software is shown to be effective for this purpose.

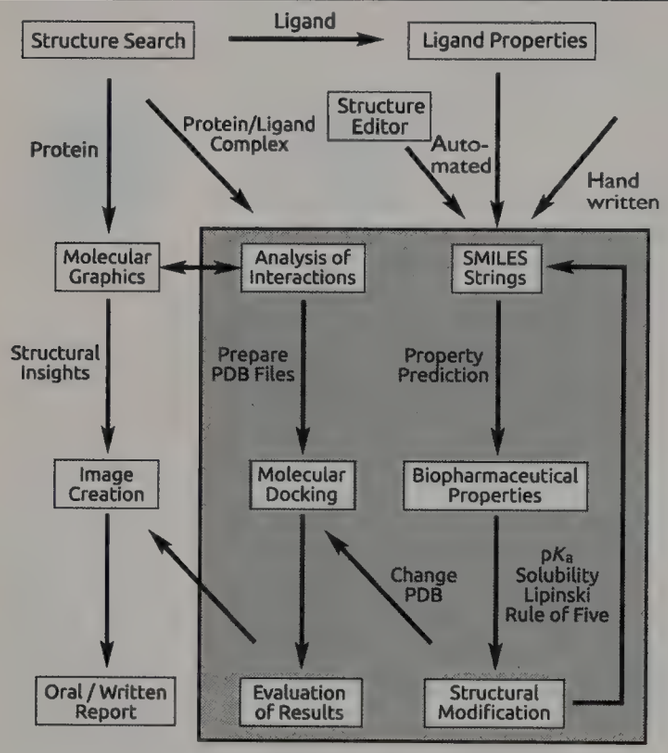

Figure 1. Flow of activities in the course. Each box in the shaded area corresponds to Web sites listed in Table 1. Each arrow indicates a hands-on activity in the course. The nonshaded area indicates related content that is not described in detail in this article.

■ COURSE DESIGN

Background

The key elements in the course are shown in Figure 1. The integration of molecular design (docking) and evaluation of biopharmaceutical properties are the primary focus of this article, as shown in the shaded part of Figure 1. Each box in the figure corresponds to a course element that can be taught in 2−4 h, with variation in the level of details. A list of URLs of Web sites associated with each course element is shown in Table 1 and the corresponding expected learning outcomes are described in Table 2. Each element is discussed in the sections below. To illustrate the teaching approach, the example of the complex of caspase-3 bound to the modified peptide inhibitor acetyl-Asp-Val-Ala-Asp-fluoromethyl ketone[26] (at the time of publication of this structure, caspase-3 was referred to as CPP32) is used. This complex provides the basis for a good example of structure-based drug design, molecular docking, and modifications to improve biopharmaceutical properties. The choice of caspase-3 was made for illustration only in this article. However, this enzyme has a well established role in apoptosis in neuronal cells and there is also emerging evidence for nonapoptotic roles of caspase-3 in neurogenesis.[27] Therefore, caspase-3 may be a potential therapeutic target.

Structure Search

The protein−ligand complex (PDB ID: 1CP3) was downloaded from the RCSB Protein Databank.[11] The procedure provides an opportunity to illustrate searching strategies on this

Table 1. URLs and Names of Web Sites Used in the Course

Course Activity[a]	Web site Name (Google Keyword Search)[b]	URL[c]
Structure Search	RCSB Protein Databank	http://www.pdb.org/pdb/home/home.do
Molecular Graphics	Accelrys Discovery Studio	http://accelrys.com/products/discovery-studio/
Analysis of Interactions	—[d]	http://ligin.weizmann.ac.il/cgi-bin/lpccsu/V_LpcCsu.cgi?PDB_ID=1CP3&Viz=Jmol&LpcCsu=LPC& [structure-specific entry][d]
Molecular Docking	MEDock Server	http://medock.csbb.ntu.edu.tw/
	Dundee PRODRG	http://davapc1.bioch.dundee.ac.uk/prodrg/[e]
SMILES Strings	Daylight SMILES	http://www.daylight.com/dayhtml/doc/theory/theory.smiles.html
Biopharmaceutical Properties	Molinspiration	http://www.molinspiration.com/
	Sparc Calculator	http://archemcalc.com/sparc/
	ALOGPS 2.1	http://www.vcclab.org/lab/alogps/
Ligand Properties	PubChem	http://pubchem.ncbi.nlm.nih.gov/
Structure Editor	SMILES Translator	http://cactus.nci.nih.gov/translate/

[a] The terms in this column corresponds to a box in Figure 1. Some entries have multiple Web sites. [b] Searching using Google (http://www.google.com/) with this phrase will locate the Web site in the first few matches (as checked in Aug 2011). [c] All URLs were accessible as of Aug 2011. [d] Accessed from RCSB Protein Databank: > Links > Analysis of Ligand-Protein Contacts (LPC). [e] Alternatively, Open Babel can be used (downloaded at http://openbabel.org/wiki/Main_Page).

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Table 2. Learning Outcomes

Course Activity[a]	Expected Outcome
Structure Search	Students are able to navigate the PDB Web site, locate a suitable protein−ligand complex, and retrieve a PDB file.
Molecular Graphics	Students understand how to open pdb files, understand the contents, manipulate a structure on the computer screen, use distance/angle tools, highlight and change atom representations, and save molecules as images, SMILES, and native formats (preserving markup).
Analysis of Interactions	Students can use the PDB Web site to identify interacting atoms and measure distances between atoms. Students can identify hydrogen bonds, hydrophobic pockets, and salt bridges formed between ligand and protein.
Molecular Docking	Students understand the process of ligand−protein docking, can extract the ligand from a ligand−protein complex pdb file, and can create pdbq files for the ligand. Students can dock a ligand to a target protein and evaluate the results for conservation of key protein−ligand interactions.
SMILES Strings	Students can convert a structure from an image to a SMILES representation and vice versa using computational tools. Students can write a simple SMILES string and modify a computer-generated SMILES string.
Biopharmaceutical Properties	Students understand the dependence of biopharmaceutical properties on molecular structure and how to influence these properties by molecular modification. Students can use Web sites for calculating key biopharmaceutical data, including pK_a, solubility, Log *P*, Log *D*, and bioavailability.
Ligand Properties	Students are familiar with PubChem as a starting point for evaluation of ligand properties.
Structure Editor	Students can use molecular editors to introduce changes into a ligand, which can then be converted into appropriate formats (SMILES) for input into other Web sites.

[a] The terms in this column corresponds to a box in Figure 1.

Web site and explore the many features and links for geometrical analysis. One such feature permits an analysis of the protein−ligand contacts (Table 1). This is used simultaneously with molecular graphics display of the complex to develop an understanding of the manner in which the ligand interacts with the protein. Identification of key interactions is required so that subsequent modifications can be made while attempting to retain key contacts. For this example, the approach is complemented by the use of a figure from the original publication, in which the contacts are described.[26] This provides a "reason" for the students to look at the literature, because there is value in the simplified published figure.

Analysis of Interactions (Molecular Graphics)

An important element in the course is the use of molecular graphics. This can be done relatively simply using just a few instructions within a graphics package such as Accelrys Discovery Studio (Table 1). This package is simple and intuitive to use and permits basic visualization of the protein and ligand and identification of ligand−protein interactions. If time permits in a particular course, a further module is added to teach molecular graphics in more detail, with more emphasis on protein structure (Figure 1). However, for a drug design course, the focus is placed on molecular association. The most effective approach is to require students to measure interactions between the ligand and the protein, which provides an understanding of contacts based on hydrogen bonding, electrostatics, and hydrophobic association. Preparation of images with coloring of key amino acids involved in the ligand interaction is also required. These activities provide a basis for modification of the ligand later in the course.

Molecular Docking

The MEDock server[28] is used as a docking platform. This Web site has the major advantage of simplicity for teaching initial principles of docking. There are alternatives, as discussed below, but the MEDock has the correct combination of efficacy and simplicity. Preparation of input "pdbq" files for input into the MEDock server is achieved using the Dundee PRODRG Web site (Table 1) after saving initial pdb files for the ligand and the protein in DS Viewer. The conversion to pdbq format also permits further discussion of molecular representation in computational chemistry because this format contains connectivity information. This is a useful educational issue because this aspect of molecular display is "hidden" behind the interface of modern molecular graphics packages. The pdbq file also includes charges, which permits a discussion of an important element of molecular association. In addition, the Dundee PRODRG server is limited to pdb files of only 300 atoms, and therefore the protein must be reduced to only the amino acids that are important for ligand binding. This is not a disadvantage in an educational context: in contrast, this requirement enhances understanding of the ligand−protein association through graphics visualization and reference to the literature.

For the docking, the ligand taken from the X-ray structure and a set of key amino acids are initially used. Rigid docking is performed for simplicity, but lecture content given in association with the docking can emphasize modern methods that allow flexibility in the ligand and protein. However, the educational goal is better served by reducing the complexity and obtaining a good answer (albeit in a somewhat idealized context) to show the students that this methodology does work. The effectiveness of this simple method is shown by comparison of the ligand geometry in the X-ray structure (Figure 2A) with the docked version of the ligand (Figure 2B). By performing the entire docking procedure, the students obtain a key skill that they can use for further docking of modified molecules, as described below.

Several points of clarification are required regarding the docking exercise. Docking was performed with a rigid conformation for the ligand (the X-ray conformation) and the protein, and this biases the results. This is clearly stated in teaching the class and it is emphasized that modern molecular docking is increasingly performed with flexible ligands and partly flexible receptors. It is also stressed that the results of the docking exercise require experimental validation; that is, they only provide a starting point for drug discovery. Regarding the choice of the caspase-3 complex for this exercise, this is used only as an example to illustrate the approach of ligand design and not because caspase-3 is currently a particularly important drug target. Also noted is that the chosen

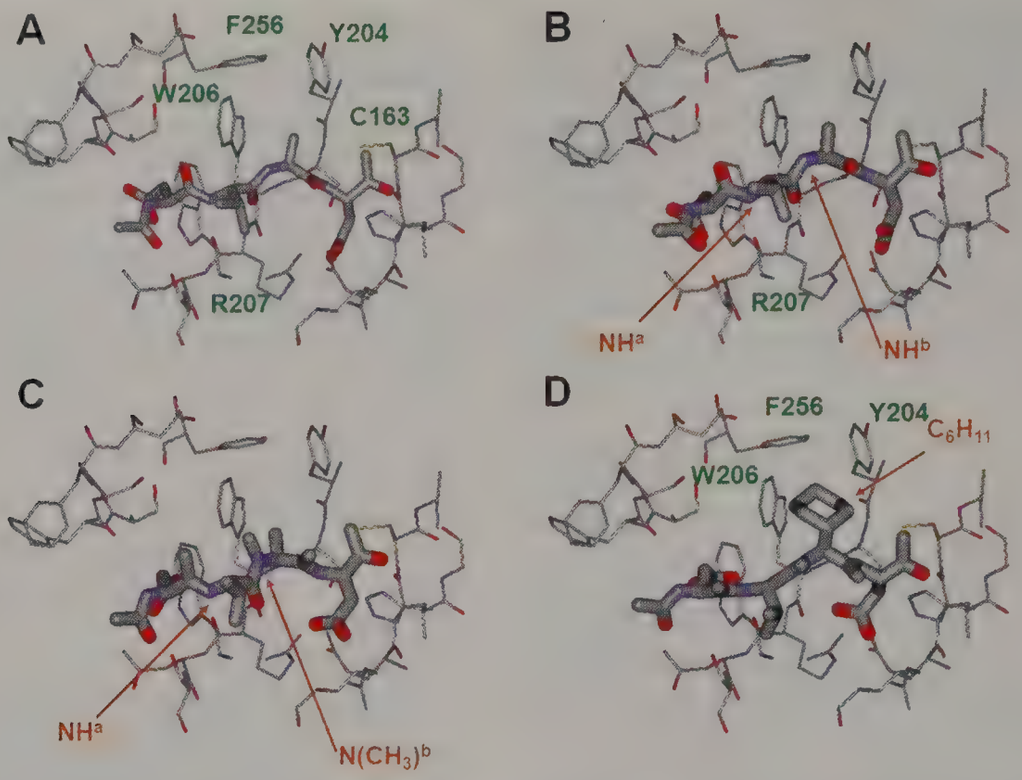

Figure 2. (A) X-ray structure of acetyl-Asp-Val-Ala-Asp-fluoromethyl ketone binding to caspase-3.[26] Only a few residues of the protein are shown. The fluorine atom of the ligand has been displaced by covalent bond formation of the ligand with Cys-163. (B) Docked version of the original ligand, showing hydrogen bond formation for NH^a and the absence of an interaction of NH^b. (C) Docked version of a ligand with N-methylation at NH^b (i.e., $N(CH_3)^b$) and replacement of two carbonyl groups with C=C bonds. (D) Docked version of a ligand with a cyclohexane (C_6H_{11}) added to the ligand in (C) to fill a hydrophobic site formed by W206, F256, and Y204. Hydrogen atoms are omitted in all images for clarity.

complex has a covalent bond between the enzyme and ligand (Figure 2). Therefore, docking was performed with a ligand that excluded the fluorine atom of the fluoromethyl group because this atom is displaced when the molecule reacts with the enzyme.

SMILES Strings

SMILES (simplified molecular input line entry specification) strings[10] are representations of molecular structure that are used as text input for software for conversion into two- or three-dimensional structure or for calculation of molecular parameters. In reverse, SMILES strings can be generated from three-dimensional structure using molecular graphics programs such as DS Viewer. Thus, a SMILES string provides an excellent medium for navigation among a variety of software. More importantly in an educational context, writing of a SMILES string requires a careful consideration of the chemistry of a molecule, including bond types, charge, and chirality.

Initially, the writing of SMILES strings is taught from first principles (i.e., handwritten strings) to increase the understanding of molecular structure and bond connectivity. However, the students are encouraged to use Web sites such as PubChem (Table 1), which provides a wealth of ligand information, including the SMILES string, and is directly accessible from the RSCB database. The use of sites for interconversion of SMILES strings to molecular structure and vice versa, including the SMILES translator site (Table 1), are also encouraged. Ultimately, the goal in teaching the background of the SMILES string is to show the students the value of this representation for navigation through computational chemistry and biopharmaceutical Web sites.

Biopharmaceutical Properties

The second part of the integrated approach is to examine the biopharmaceutical properties of the original ligand and modified ligands. Having established strategies for obtaining a SMILES string, these strings are used in several Web sites to obtain properties associated with Lipinski rules, solubility, ionization (pK_a), potential degradation, metabolism, active transport, and bioavailability. The extent of this approach depends on the available time in the course. For a relatively basic approach, using the Molinspiration Web site (Table 1) is favored because this site is simple to use and provides a straightforward overview of the Lipinski properties of a molecule. This Web site can be accessed with a SMILES string or through simple structure building using an intuitive molecular editor.

Snapshots from the Molinspiration Web site output for the original ligand are shown in Figure 3B, based on input of the SMILES string in Figure 3A(i). These kind of results provide an excellent basis for understanding the biopharmaceutical issues associated with ligand design. Most of the properties violate the Lipinski rules, including the very negative LogP (miLogP), and the related high numbers of hydrogen bond acceptors (nON) and donors (nOHNH). This provides a good basis for discussion of the likely difficulty of oral delivery of this agent, based on its probable poor absorption properties. In addition, the number of rotatable bonds (nrotb) is high, and this allows discussion of potential affinity problems and the need to build in rigidity in the molecular structure. Further analysis of the molecule is possible using other Web sites (Table 1), such as the ALOGPS 2.1 site for calculation of solubility and the SPARC site for evaluation of ionization and other properties, and through discussion of potential in vivo degradation (because the molecule is peptidic). However, the Molinspiration site provides sufficient detail for discussion in an introductory course.

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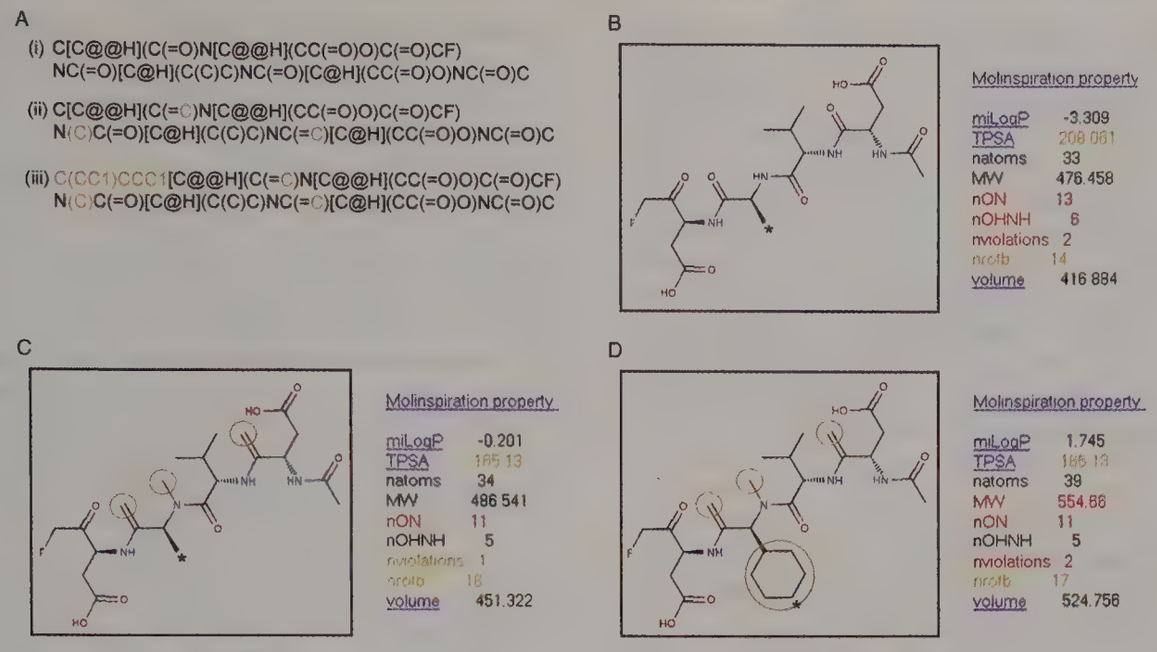

Figure 3. (A) SMILES strings of (i) the original ligand in Figure 2A,B, (ii) the modified ligand in Figure 2C, and (iii) the modified ligand in Figure 2D. Modifications to the SMILES string of the original ligand are shown in orange. (B–D) Molinspiration output showing biopharmaceutical properties for the three ligands. The asterisk on each ligand (not present in the Molinspiration output) indicates the C atom at the start of the respective SMILES string. Each ligand was redrawn from the Molinspiration output to produce a similar orientation. Changes from the original ligand (B) are indicated by orange circles in (C) and (D) and correspond to the changes in the SMILES strings shown in orange in (A).

Structural Modification and Evaluation of Results

At this stage, the students have developed the requisite methodological skills and have sufficient knowledge of a particular protein–ligand complex to permit modification of the original ligand, with the goal of improving the biopharmaceutical properties while maintaining the binding affinity of the modified ligand. They have an understanding of molecular graphics, basic docking skills, the ability to write and modify SMILES strings, and an appreciation of favorable biopharmaceutical properties. They are now ready to integrate these activities in an exercise that mirrors the drug design process at the research and development level.

To illustrate this procedure, two modifications of the original ligand based on evaluation of the original complex (Figure 2A) or the similar docked version of this complex (Figure 2B) are used. An aspect of the binding is the use of one peptide bond N–H in the ligand (NH^a in Figure 2B) to form a hydrogen bond to the backbone carbonyl group of R207, while in contrast, a second ligand N–H (NH^b in Figure 2B) appears unimportant in the ligand–protein interaction. This suggests that N-methylation of this peptide bond may be allowable from a binding perspective, while also improving the biopharmaceutical properties (reducing the hydrophilicity). This change (and isosteric replacement of two carbonyl groups in the ligand with alkenes) was implemented through modification of the ligand in DS Viewer. The resulting PDB file was processed and docked using MEDock (Figure 2C). The SMILES string for the new ligand (Figure 3A(ii)) was obtained by modification of the original SMILES string, and then used in Molinspiration to compute the biopharmaceutical profile (Figure 3C).

This exercise provides a valuable learning experience and is accomplished by the students in a relatively short period of time. The results are "real" and relatively easily interpretable. It is clear that the key interactions of the original ligand with the protein have been maintained in the docking of the modified ligand (Figure 2C) (these interactions can be quantified), whereas some of the problematic Lipinski properties have been improved. This can be developed further. For example, a hydrophobic site formed by W206, F256, and Y204 seems to be present in the ligand binding site, and it is possible that this could be exploited by addition of a large hydrophobic group to the ligand (a cyclohexane is used in this example). Further docking and evaluation of biopharmaceutical properties gives the results shown in Figures 2D and 3D, respectively.

Additional Teaching Elements

The choice of a given complex can be made such that key principles can be illustrated through the procedure of design and biopharmaceutical evaluation. In addition, the use of a particular structure allows other principles related to the structure to be discussed. For the caspase 3–Ac-DVAD-fmk complex, a brief background discussion of the caspase cascade in apoptosis is provided. Discussion of a "suicide inhibitor", given the formation of the covalent bond to Cys-163 in the protein, is also possible This discussion requires presentation of the basic catalytic mechanism of a cysteine protease, and how this mechanism is exploited in the design of the inhibitor. These important principles become clearer and perhaps of more interest to the student because they have become acquainted with the protein and the inhibitor. Clearly, these teachable opportunities will vary from complex to complex, but an interesting background presentation should be possible for each complex.

■ DISCUSSION

The approach described above can be used in several teaching contexts. Variants of the approach have been used in short intensive courses (7 h per day over 5 days) or in semester-long courses. In the first setting, the material is taught through short lectures (typically of about 30 min) followed by directed computational exercises. These exercises work best in small groups of students (three or four) and with floating assistance from the lecturer and assistants. Given the relatively short time

availability, there is a need to move the exercises forward and ensure that each student or group is moving at the same pace.

The course has also been taught at the introductory graduate level over a semester. The material does not differ, but the problem is given to the students as a case study, in which there may also be an additional element to mimic an industrial setting. This can involve assignment of an enzyme class, with a small group of students asked to research this enzyme class, determine its relevance to disease, and then identify a target (with a solved X-ray structure) prior to engaging in the activities described above. This clearly requires much more time, but the case study can be performed as an out-of-class assignment while the lectures in the course provide background on the methods. In this setting, it is also possible to include several student presentations of the findings.

The procedure can also be presented as a lecture or demonstration without significant hands-on work by students. This includes an introduction of basic principles and discussion of some of the more detailed ideas associated with the complex. A class time of 6 to 8 h is needed for this approach. Some preparation would also be required, particularly for building and running the files for docking. This approach may be appropriate for a graduate-level course in which drug design is one of several components and could be given by a single lecturer. It has the disadvantage of not allowing students to use the software and is also limited by the focus on one complex only. However, the approach may still be more effective in inspiring student thinking, compared to a purely didactic description of the principles of drug design.

An important element of this article is that the course (in any of the above settings) can be performed entirely with free and Internet-based applications. The availability of such extensive and effective software is a remarkably positive aspect of the Internet and a testament to the generosity of the authors of this software. This availability also permits the course to be mobile both geographically and in a computer platform-independent manner. More sophisticated software applications are available and may be appropriate in some educational settings. However, these require greater technical understanding and more time for adequate interpretation of results. For example, Autodock Vina[29] is an open-source molecular docking program that provides a platform-independent method for docking ligands to target molecules. The interface to Autodock Vina is intuitive and the steps required to obtain docking results can be taught through a video tutorial. Autodock Vina provides greater flexibility and customization than is afforded with MEDock. Having several programs that accomplish the same goals with different depths allows educators to tailor the curriculum to the audience or time constraint.

The curriculum could also be supplemented with commercial programs for functions that are not adequately filled by open-source software. ACD Labs ADME suite[30] can be used to predict the probability of a ligand being a substrate for cytochrome P450 metabolism and *P*-glycoprotein efflux. GastroPlus from Simulations Plus[9] can be used to predict absorption and bioavailability of molecules and establish a direct relationship between structure and in vivo drug performance. These programs allow students to appreciate cutting edge ADME prediction methods. Rational modification of the structure can be achieved with feedback on whether a change was positive based on drug absorption profiles that include many factors influencing bioavailability. This is valuable for extending the course to a more sophisticated discussion of drug design and delivery, but clearly requires more time and a wider series of lectures on issues such as drug metabolism and transport. Other related areas can also be included in the course, at the discretion of the lecturer and within the time available. In particular, more discussion of structural biology can be included as an additional module (Figure 1), with a focus on protein and DNA structure. A further possibility is extension of the drug design element of the class to include discussion of QSAR and pharmacophore methods, perhaps based on the results obtained from the docking exercise.

The main concern in developing new modules as extensions of the drug design focus is to maintain the hands-on computational approach. This may be the most important aspect of the course. First, the computational approach is helpful in overcoming difficulties with language, if the class contains students with a first language that differs from that of the instructor. The course has been taught in several international settings and the effectiveness of the computer-based approach is evident. Only this anecdotal experience is presented and there seems to be an absence of academic literature addressing this issue. After performing a few exercises in docking, SMILES string development, and interaction with key Web sites, students become proficient in the techniques. The methods are no longer a barrier to obtaining output. The output also has a sense of "belonging" to the student because the design of the new ligand was based on individual or group-based ideas. The end point is understandable and achievable, and this produces a greater desire to learn and recognize the creativity of the science behind the drug-design process. This is a key to developing future scientists who will be effective in this area and may be able to reverse the current trend in the decline of blockbuster drugs emerging from the pharmaceutical industry.[31]

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Several versions of this course have been offered in the graduate program of the Department of Pharmacology and Pharmaceutical Sciences at USC and in international courses in association with universities in Taiwan and Thailand. We are grateful to all the faculty and teaching assistants who have contributed to these offerings of the course.

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ARTICLE

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The Development and Implementation of a Problem-Based Learning Format in a Fourth-Year Undergraduate Synthetic Organic and Medicinal Chemistry Laboratory Course

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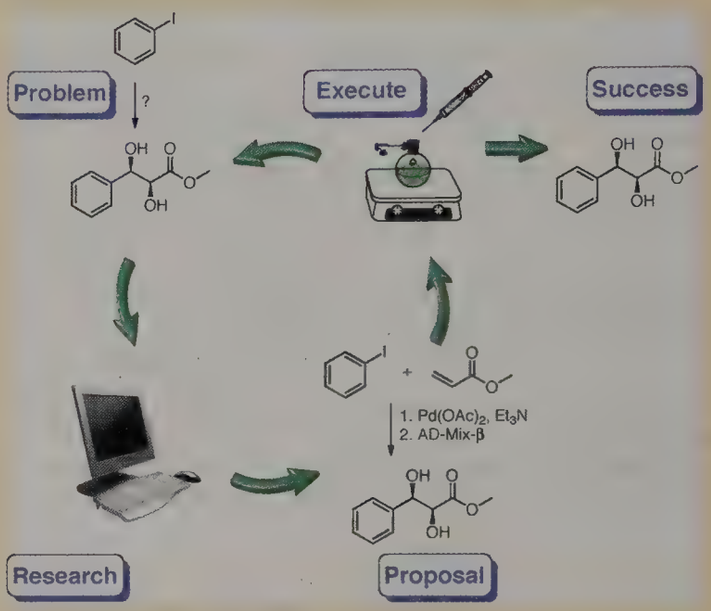
Supporting Information

ABSTRACT: The fourth-year undergraduate synthetic organic and medicinal chemistry laboratory course at the University of Ottawa was transformed from a traditional laboratory format to a problem-based learning (PBL) format. Authentic problems were developed that closely resembled the types of problems that scientists regularly confront. The development, implementation, and challenges of the fourth-year organic and medicinal chemistry laboratory course taught in PBL format are discussed, as well as results and feedback from the course.

KEYWORDS: Upper-Division Undergraduate, Curriculum, Laboratory Instruction, Organic Chemistry, Collaborative/Cooperative Learning, Inquiry-Based/Discovery Learning, Problem Solving/Decision Making, Medicinal Chemistry, Student-Centered Learning



Problem-based learning (PBL) is an educational approach that uses complex, real-world problems to motivate students to identify and research the concepts and principles that they need to know to devise a solution to the problem.[1] Students typically work in small groups, and while learning course content, other key abilities are developed including the ability to think critically; find, evaluate, and use appropriate learning resources; communicate effectively; and work independently of the instructor. Because the problems given are complex and realistic, the path to the solution is not always evident, and there is often more than one way to solve a problem.

A critical-thinking ability is essential for a graduate in the sciences. One definition is given by Van Gyn and Ford: "The self-regulated deliberations on a challenge or problematic situation that involve consideration of generated or selected alternatives directed towards evaluative judgments. Judgments are based on criteria, which provide justifications for the conclusion."[2] Three integral dimensions are also included in the authors' model of critical thinking: intellectual habits, intellectual deliberations, and a reflexive disposition.[2] It is essential that critical-thinking abilities be developed by the time a student graduates and the teaching of these abilities must therefore be incorporated into science courses.

A traditional laboratory course typically gives students a procedure to follow to make a specific target compound. Students follow the instructions, much like following a recipe.[3] In this process, students learn essential laboratory techniques such as the manipulation of air-sensitive compounds. They also learn the mechanistic details about the reaction in question, usually after the experiment when they are writing a laboratory report.

In a traditional laboratory experiment, the students do not develop important critical-thinking skills. Kelly and Finlayson have discussed the traditional laboratory format in depth and have explained why there might be a need to change.[3] It was important to us that the students learn how to select an appropriate experimental technique, how to decide whether a given technique is required (e.g., whether the compound is air-sensitive), how to evaluate different experimental strategies, and how to plan a complete experiment or project. Problem-based learning was selected as an appropriate method through which students could learn important critical-thinking skills and experimental abilities.

The implementation of PBL has been described in lecture[4] and laboratory[4e,5] courses and this learning method has often been employed at the first- or second-year undergraduate level. In lecture courses, for example, Dods described specific and engaging problems that were incorporated into a biochemistry course.[4a] Cannon and Krow incorporated synthesis projects into upper-level organic chemistry lecture courses, in which students,

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Table 1. Problems and Associated Learning Objectives

	Title	Learning Objectives
1	Separation of a mixture	By the end of the problem students will: be able to identify and execute an appropriate method for the separation of compounds. be able to analyze the purity of each compound. be able to propose a structure for each compound. report their findings in an acceptable scientific format. Note: this first problem served as a review of NMR, chromatography, and other separation methods and as a first step in working through the PBL process.
2	Correction of an ineffective procedure	By the end of the problem students will: be able to draw the mechanism for a Sonogashira reaction, identify key characteristics of that reaction (appropriate catalyst and co-catalyst choices, solvent, base, relative reagent quantities), and design an experimental procedure accordingly. be able to recognize a target as a reasonable candidate for a Sonogashira reaction. Demonstrate correct technique for the handling of air-sensitive reagents.
3	Design of a short synthesis of a biaryl compound	Given a target biaryl compound, students will: be able to design a short, efficient synthesis. make decisions with respect to the cost and availability of reagents, in addition to the length of the synthesis.
4	Enantioselective synthesis and *ee* determination	Given a target molecule, students will: be able to identify appropriate reagents to carry out a Heck reaction. be able to accomplish an enantioselective dihydroxylation. be able to identify and use an appropriate method to determine enantiopurity. be able to describe the difficulties associated with identifying the major enantiomer formed.
5	Design of a drug synthesis	Given a drug target, students will: be able to design and carry out a synthesis of a common drug or drug analogue. be able to describe how a given route could be more suitable for process chemistry, medicinal chemistry, or academia.

in groups, presented a literature synthesis and subsequently their own synthetic schemes to their class.[4e] For laboratory courses, Kelly and Finlayson described the development and implementation of first-year analytical and general chemistry PBL modules.[3] Ram described the implementation of PBL with authentic chemistry problems in a second-year analytical chemistry laboratory to motivate students.[5a] The book entitled *Experimental Organic Chemistry* has a section on multistep syntheses that explains to students how to design synthetic sequences, gives prelaboratory questions, and has a list of examples of synthetic targets that are appropriate to the introductory level.[6]

Herein, the transformation of a fourth-year undergraduate synthetic organic and medicinal chemistry laboratory course from a traditional format into a problem-based learning format is described. Important aspects and considerations that are addressed include (i) the development of effective problems suitable for an advanced organic chemistry laboratory course; (ii) workshops that were developed to explicitly teach the problem-solving process as it pertains to advanced organic chemistry and that were important to setting students up for success; (iii) the typical problem solving process and student proposals; (iv) the challenges encountered, particularly those that were specific to an advanced organic chemistry laboratory, as well as the associated recommendations; (v) student opinions; (vi) student results; and (vi) instructor and teaching assistants opinions.

■ THE COURSE

This fourth-year laboratory course entitled "Synthetic and Medicinal Chemistry Laboratory" is offered to students in the biopharmaceuticals science program. Typical enrolment is approximately 12 students. A PBL format was first implemented in the fall of 2008. The 12-week course, which involved one 6-h laboratory period per week, began with an introductory session and workshops. Over the course of the remaining 11 weeks, the students solved five problems, of which two required multiple weeks of experimentation, and performed one to two traditional lab experiments.

■ DEVELOPING LEARNING OBJECTIVES

Overall in the course, it was expected that students learn the course material in greater depth than students in past courses, that students become independent learners, and that students improve their critical-thinking skills in areas that included chemistry research, synthetic design, experimental execution, and the analysis of results. Each problem in this course was designed to satisfy a specific learning objective or objectives, which are outlined in Table 1.

■ CREATING PROBLEMS

There were a number of considerations when developing the problems. From a general point of view, problems were developed that (i) provided an opportunity to satisfy the learning objective(s), (ii) were complex and potentially had multiple possible solutions, and (iii) were realistic. It was also necessary to consider (i) how much time the students would need both for research and for experimentation in the laboratory; (ii) whether to share the learning objectives with the students, and if so, when; (iii) whether a story-line should be included as is common to

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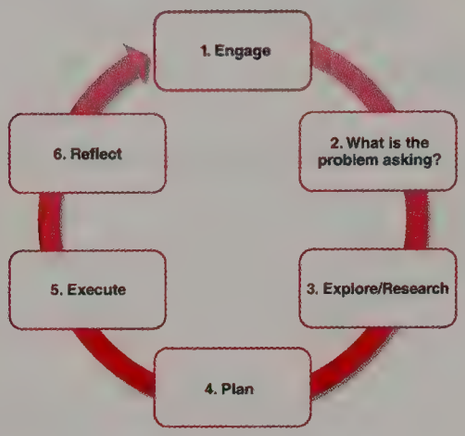

Figure 1. The McMaster six-step problem-solving strategy.[8]

PBL problems; and (iv) how to present the problem (as a task, series of questions, etc.).

The ideal problems resembled those encountered by chemists in a professional environment and for this particular course included (i) the correction or improvement of an existing protocol; (ii) the development of methodologies while taking into account factors such as cost, safety, and efficiency; and (iii) the synthesis of new compounds. The titles of the problems developed are shown in Table 1 and the problems themselves can be found in the Supporting Information. The problems were designed with a gradient of difficulty so that students could familiarize themselves with the PBL process with more straightforward problems initially.

A fourth-year undergraduate student researched and tested a variety of possible solutions that students might propose prior to the first PBL course. While not every possibility could be examined, this testing provided the opportunity to evaluate each problem from a student's point of view.

A manual was also created for the course's teaching assistant (TA) that contained solutions proposed by previous students, guidance for the TA in terms of potential problems that students might encounter, and the common problems or shortcomings seen in past proposals. This manual was a valuable means of communicating information between TAs from course to course and was continually updated.

■ SETTING STUDENTS UP FOR SUCCESS

The PBL method was completely new to the students and a number of skills and attitudes were identified as essential for student success using this new method. Skills that were considered included creativity, problem-solving, teamwork, stress management, time management, self-assessment, self-directed learning, understanding expectations, and critical-thinking skills. From this list, the most crucial skills for this course were identified. To be successful in this PBL course, students had to be able to cope with change, already be effective problem-solvers, and understand the course expectations.[7] The workshops, which were created accordingly, are described below.

For students to cope with this change of learning format that seemed drastic to many of them, a discussion was held during the introductory session of the course that described the process of coping with change and also referred them to the student resource: *How to gain the most from PBL*.[8] Three workshops were also conducted in the introductory session, each about 1.5 h in length that covered problem solving, group work, and course expectations.[9] In the problem-solving workshop, students learned and practiced an explicit problem-solving process. The process that they followed was the six-step McMaster strategy shown in Figure 1.[8]

The students practiced this process using a general problem that was unrelated to chemistry so that they could focus on developing and practicing the process skills. That workshop concluded with a comparison of the problem-solving strategies used by novice and expert problem solvers in which it was noted that expert problem solvers tended to spend significantly more time in the first three steps of the process, defining the problem, and regularly went to step six, reflection. In contrast, novice problem-solvers spent relatively little time exploring the problem (steps 1−3) and went quickly to steps four and five, spending little, if any, time reflecting. Developing these process skills helped students work more efficiently and were very useful when stumbling blocks were encountered when solving problems.

The students enrolled in the first section of the course given in the PBL format demonstrated that they had little experience and ability conducting scientific research. They were inexperienced both in knowing where to find information and how to assess it for relevance, reliability, and so forth. In this first problem-solving workshop, students worked collaboratively to identify relevant databases and search engines. Using this list as a starting point, a discussion was facilitated relating to the reliability and relevance of the information procured from various sources that included peer-reviewed journals, newspaper articles, reviews, books, Wikipedia, and search engines or databases such as SciFinder and Google. In the first iteration of the course, the students did not have opportunity to practice or apply this theory until they were confronted with the first problem. To ease the transition to PBL, a second section of the problem-solving workshop is planned for future years, which will ask students to do basic chemistry research, also in the PBL format, and will prepare them for the actual problems that they will encounter in the rest of the course. Several students at the end of the course commented that they greatly valued this opportunity to learn to do scientific research and apply their findings in the laboratory. Most of them had never done this type of research before.

In the workshop on group work, students identified issues that tend to arise when working in groups as well as anxieties that they had about group work. They then developed a set of guidelines, such as a contract, that they agreed to follow during the course. Because of the small class size, there were typically two to three students per group.

In the final workshop, which dealt with expectations and assessment, the expectations of the course were described to students and from there the students developed their own evaluation scheme for the course. The expectations included the elements required in the pre-experiment proposal, the lab notebook, and the post-experiment report, plus the requirements for the one to two traditional laboratory reports. As another option, in one course, students decided to include peer evaluations as part of their assessments in order to acknowledge the work contributed (or not) by each group member.

■ HOW WAS A TYPICAL PROBLEM SOLVED?

Students were presented with a problem and developed a proposal that had to be approved before they were allowed to execute their proposal in the laboratory. In solving the problem,

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they had to record their progress through the problem-solving process and include the following items in their proposal:

- Background on the reactions to be performed (mechanism and general details)
- Reaction schemes and explicit experimental procedures
- Justification of choice of method or route
- Table of reagents
- Safety data for each reagent including how to handle the compound (air sensitive, etc.), how to dispose of the compound, and what to do if a minor spill occurred
- A description of how to perform any new technique
- Research record: problem-solving process taken, Web sites visited, references to articles

Most proposals required some type of feedback or correction. Depending on the situation, the proposal could either be approved immediately and the student could proceed directly to the lab or the student would have to return to do additional research and resubmit the proposal. Once the student's or a group's proposal had been approved, he, she, or they could go into the lab and try his, her, or their proposed experiment(s). Everyone was working on each problem in the same order, although students could choose to move ahead and overlap problems (e.g., someone could start a new reaction while another reaction was running or before purifying a previous reaction).

If the students ran into roadblocks along the way, they were responsible for finding solutions. This could mean changing solvent systems for a column or changing reaction conditions for the experiment—this sometimes involved additional research to determine what changes were most likely to be effective, a process that is not often encountered in a traditional laboratory course. During this process, consultation and collaboration with their peers, even outside of their own group, was encouraged.

A typical lab report was not required for the PBL sections of the course, but a description and analysis of the results and a reflection were required. Following the experiment, students supported their results primarily using the analysis of their NMR data (Bruker AVANCE 300 NMR spectrometer).[10] The final report, submitted by each student individually, also included a reflection of the entire process, which is an integral part of the six-step problem-solving strategy. A future course could also include an abbreviated research paper, including appropriate formatting, to tie together everything that the student has learned.

■ CHALLENGES

A number of challenges were faced in the initial implementation of this course that were fairly specific to a laboratory course. These challenges are discussed below and include the planned solutions.[11]

Initially, student proposals were too simplistic or incomplete. Students did not anticipate many of the things they would have to do in the lab. This might be something as simple as not considering whether a reaction needed to be performed under an inert atmosphere, what type of workup or purification would be appropriate, or designing an experimental procedure that lacked key experimental details. At times, students did not take into account the cost, safety, or stability of the reagents that they had selected, which had a significant impact on the quality of their proposal.

The reality of this type of course is that constraints such as budget, safety, reagent availability, and time limit what the students are actually able to do in the lab. Students initially were trying to guess at the reagents that would be available so that they would not later have to make changes to their experimental

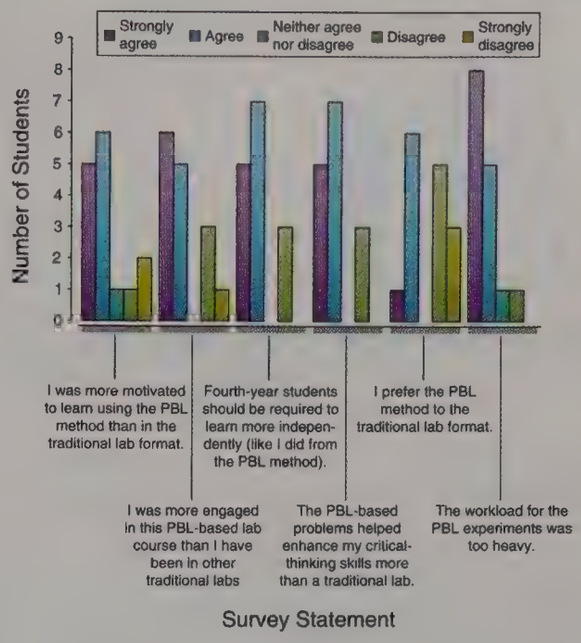

Figure 2. Student opinions regarding the PBL format ($N = 15$).

procedures rather than trying to develop the best possible solution. It was difficult for students to understand that there was not a single "correct" answer. Although this ambiguity was frequently unsettling, it encouraged them to understand the given problem more fully and evaluate multiple possible solutions.[2] There are different ways to approach this type of issue, such as giving the students a list of the available reagents or telling them in which journal(s) they were likely to find an appropriate experimental procedure. It was important, however, to leave the possible solutions as broad as possible in order to best mimic the work of a professional chemist who would have to consider all of the aforementioned factors.

As such, student proposals were evaluated independently of the reagents and equipment that were actually available in the laboratory. The merit of the proposal, which was evaluated by the teaching assistant or the professor, was based on efficiency, cost, and safety in addition to any specific requirements of individual problems. For future iterations of the course, in the event that it is not possible for the student to execute his or her proposal in the laboratory, the student will be given an experimental procedure to use. It must be noted that, despite a sometimes wide variation in student proposals, many could nevertheless be attempted in the lab, all depending on which reagents were available.

By approaching the issue of simplistic or incomplete proposals in this way, the problems were kept realistic. Evaluating the proposals according to their merit independently of the reagents and equipment available in the laboratory allowed the students to recognize the value in their proposals.

Transference (i.e., copying of answers from one year to the next) was another concern. To deal with this, minor variations were made to each problem between courses and a bank of problems has been developed so that a rotation will continually be possible.

Inevitably, in science, experiments fail. Unfortunately, students are conditioned with traditional lab experiments to expect a "good" result every time. Some students became discouraged in and disengaged from the experiment if their proposal or reaction did not work or if it gave unanticipated results. For example, one student attempted a Sonogashira reaction using water as the solvent and could not isolate product from her reaction. She

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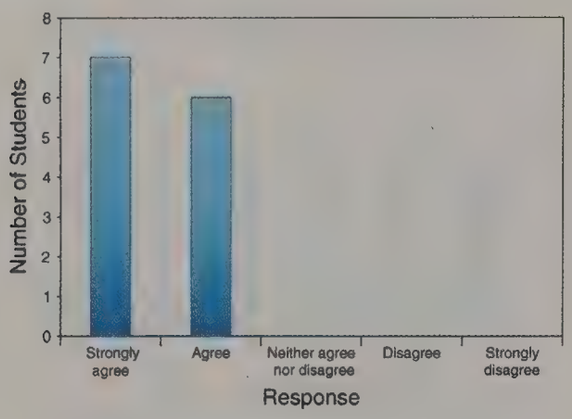

Figure 3. Student responses to the statement: "I learned more using the PBL method than with a traditional laboratory format."

became frustrated that her reaction did not work as described in the literature and abandoned the reaction. Future introductory workshops will address this issue and will ask students to develop specific expectations and establish a process to follow should a reaction not work as anticipated. In essence, the intent was for the students to learn to collaborate and optimize reactions in parallel as much as possible and to realize that a "failed" reaction is just as much a part of the learning in science as is a "successful" reaction. Although the students did have the time to repeat and optimize reactions, there was not a large grade incentive for doing so. Only a few points were accorded for obtaining the final product; a majority of marks were given for the proposal and process. This could be changed in the future to increase the value of obtaining the desired product in a high yield.

■ STUDENT OPINIONS

Feedback was collected from students in the form of surveys in which Likert-scale and open-ended questions were asked. A cross-section of the results is shown in Figure 2. The feedback from students was interesting. Overwhelmingly, students felt, as compared to a traditional laboratory course, that they had learned more and more deeply, that their critical-thinking skills had improved, that they were more engaged in the course, and that fourth-year students should be required to learn more independently as they were required to do in the PBL section of this course. Conversely, students felt that the workload for the course was too high and most preferred the traditional lab structure to the PBL course format.

In the comments made early in the course relating to workload, many students asked that they be given more information or hints in order to find the "correct" procedure; two mentioned that they did not "like" spending hours in the library trying to find an appropriate procedure. Placing more value on the proposal as an entity independent of the laboratory largely alleviated students' anxieties with respect to finding the "right" procedure. The reality is that both research and learning take time,[12] and this time spent is extremely valuable in terms of student growth, even though it might not translate into an actual experiment or specific research being used in the laboratory. One student's comment highlighted the fact that learning or working in a new way does take more time at first: "I think the only reason why it [the workload] was too heavy at first is because fourth-year students are not used to seeing PBL methods and going out and doing their own research to solve a problem. After the first month or so I got used to PBL and the work load had seemed average at that point." In fact, the workload was comparable to the traditional laboratory once students became comfortable with research. There was more time required than usual to prepare the proposal (in comparison with a traditional prelab quiz); however, there was much less time required at the end on the experiment(s) (in comparison with a traditional lab report).

In addition to workload, an important component of that student's comment was that students are not used to doing their own research to solve a problem. It is essential that students completing a degree in science be able to conduct research and certainly by the fourth-year level. Many students subsequently commented about how much they had improved their independent research abilities, which was one of the key objectives in the course.

Comments relating to student preference for one method over another showed that students were more comfortable in the traditional format and felt that format required less work, even though a detailed formal lab report was required following the experiment. This resistance or aversion to change has been commonly reported.[2,8,11] Despite the stated preference for the traditional format, many students made positive comments related to the independent research that was part of the lab, such as the following: "I liked the independent research that was encouraged in the course, and how I dramatically improved my research skills." The fact that students recognized that their research and independent learning skills have improved highlights the value of the PBL method. Additionally, the students unanimously stated that they learned more using the PBL method (Figure 3).

Table 2. Student Exam and Final Grades

Semester - Year	Format	Class Size	Mean Exam grade (%)	Mean Final grade (%)
Fall - 2007	Traditional	12	78	73
Winter - 2008	Traditional	13	74	81
Fall - 2008	PBL	3	83	77
Winter - 2009	PBL	12	81	77

■ STUDENT RESULTS

The exam and final grades of students who had taken the course in PBL format were comparable to those who had taken the course previously (Table 2), showing that the PBL students learned the course content at least as well as the students who had taken the course in the traditional format. The exams were very similar in each course; only slight modifications were made to reflect the actual experiments performed in each year while keeping the question types the same.

■ PROFESSOR AND TEACHING ASSISTANT OPINIONS

Having made the modifications in the course to run in the PBL format, the professor found this format is extremely beneficial to students and that these benefits for students go far beyond learning specific reactions. The biggest challenges were in problem development, seeking characteristics that were previously described. Developing workshops, designing problems, and testing problems were the most time-consuming aspects of the conversion to PBL. Evaluating the proposals (either by the professor or by the teaching assistant) for feasibility was straightforward and was not particularly time-consuming, and certainly not more than evaluating a traditional laboratory report. Marking

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the proposals and making sure every required component was included took more time; however, these tasks were done by the teaching assistant and were equivalent to marking end-of-lab reports. The first time that the course was run, there were regular discussions when questions came up, such as should a student be allowed to try a route that was likely to fail, how to evaluate a proposal that included more complex reactions than required, and so forth. Certainly, having extremely experienced teaching assistants, as was the case when this PBL format was introduced, is an enormous benefit.

Both the teaching assistants and professor had to become accustomed to guiding students when they asked questions as opposed to immediately telling them the answer. It was also important to recognize that students should be free to attempt nonstandard reactions, provided that they would not pose a danger to themselves or to their colleagues.

When incorporating PBL into a course, it is also possible to begin by adding only one problem at a time and slowly replacing or modifying existing experiments. This method can enable both the students and the instructor to gradually become accustomed to the PBL format. Indeed, a subsequent professor for this particular course has chosen to use a few PBL problems interspersed with traditional lab experiments to keep the course more straightforward for the students.

■ CONCLUSIONS

A problem-based learning format was successfully developed and implemented in a fourth-year undergraduate synthetic organic and medicinal chemistry laboratory course. Students at this level have the background required to research and solve advanced and complex problems. To solve these realistic problems, the students followed the McMaster six-step problem-solving strategy. They first identified and explored the problem, then conducted scientific research to develop a proposal of a solution. Upon approval, the students executed their proposal in the laboratory. Through the analysis of the results, the students determined to what extent their strategy had been successful and had the opportunity to improve on it in the laboratory.

The course objectives have been met and the benefits to students outweighed the challenges. The students learned the course material and laboratory techniques at least as well as students who had previously taken the course in the traditional format. This was evidenced both by final exam results and by the unanimous student agreement with the statement "I learned more with the PBL format than I would have in a traditional lab format." Most notably, the students improved their abilities to learn independently and to think critically, as evidenced by the proposals submitted before each experiment, by their work in the laboratory, and by their ability to analyze their results. Research skills, critical-thinking abilities, and independence in the laboratory, which were required for solving the problems, were newly acquired in this course by the vast majority of students and are essential abilities needed for future independent learning whether in graduate school or in their future careers.

■ ASSOCIATED CONTENT

Supporting Information

Student lab manual. This material is available via the Internet at http://pubs.acs.org.

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ARTICLE

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History and Epistemology of Science in the Classroom: The Synthesis of Quinine as a Proposal

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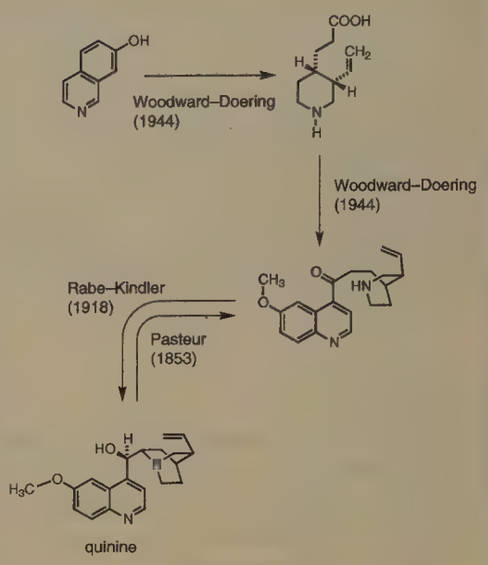
Supporting Information

ABSTRACT: The history of the quinine synthesis can be used as a case study to emphasize that science is influenced by social and historical processes. The first efforts toward the synthesis of this substance, which until recently was the only treatment for malaria, were by Perkin in 1856 when, trying to obtain quinine, he synthesized mauveine. Since then, the quest for the total synthesis of quinine involved several characters in a web of controversies. A major step in this process was made in 1918 by Rabe and Kindler, who proposed the synthesis of quinine from quinotoxine. Twenty-six years later, after obtaining the total synthesis of quinotoxine, Woodward and Doering announced the total synthesis of quinine. However, the lack of experimental details about Rabe and Kindler's process, associated with Woodward and Doering's failure to reproduce it, raised a series of doubts about the synthesis. Stork and colleagues questioned the veracity of the experimental data and even the scientific reputation of the involved researchers. Doubts remained alive until 2008, when Williams and Smith reported, not without reservations, the reproducibility of Rabe and Kindler's protocol. The scientific knowledge as a social and historical development, its legitimating process, and the absence of neutrality in science constitute aspects that can be discussed from this case study, providing significant contributions to science education, in particular, to the initial or continued training of chemistry teachers.



KEYWORDS: Graduate Education/Research, Upper-Division Undergraduate, History/Philosophy, Organic Chemistry, Chirality/Optical Activity, Drugs/Pharmaceuticals, Synthesis

In recent years, science educators have been concerned with the diversity of information available and its means of dissemination, as well as with the persistence of poorly informed conceptions of science, scientists, and scientific knowledge among students and teachers in all educational levels. Research articles draw attention to the prejudicial impacts of such misconceptions on both the intellectual development and the professional performance of these individuals. A review of the literature (from 1984 to 1998) on science education was made by Pérez and co-workers,[1] addressing the distorted conceptions of science manifested by teachers.[2] They observed the persistence of conceptions that describe scientific knowledge as inflexible, elitist, dogmatic, and accumulative.

Considering the influence of teachers' conceptions of science on their pedagogic strategies,[3] it is not surprising to find that the same conceptions are prevalent among students at basic, intermediate, and higher educational levels, as demonstrated by Alonso and Mas,[4] Petrucci and Ure,[5] Kosminsky and Giordan,[6] and Dagher.[7] Still more concerning is the recognition of the presence of these conceptions in the discourse of the next generation of researchers, that is, graduate students who will be scientists and university lecturers in the near future.[8]

Research results have increasingly favored the inclusion of history and philosophy of science as components of science curricula.[9,10] History and philosophy of science may help the understanding of both scientific knowledge and its creation process, which are currently considered equally important. Various justifications have been presented in the literature for the inclusion of historical material in science teaching, including the perception of science as a human undertaking that evolves throughout history, as well as its contribution to significant learning as the classes become more interesting and reflexive.[11] Maldaner (ref 3, pp 62−63) suggested that

> According to current practice, in which the knowledge presented in scientific disciplines is taken to be truth and the expression of an external objective reality, what emerges are tacit conceptions, based on beliefs and formulations, that provide no critique of what science might be or its significance in the contemporary world.

For Matthews, who emphasized the importance of the history and philosophy of science in the context of science teaching, and especially of the training of teachers, resistance to the "mass production" of naïve and uncritical students and professionals

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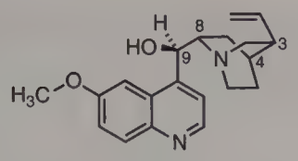


Figure 1. Structural formula of quinine.

would be greatly assisted by adoption of this approach in science teaching (ref 10, p 213), because

> A historically and philosophically literate science teacher can assist students to grasp just how science captures, and does not capture, the real, subjective, lived world. An HPS [history and philosophy of science]-illiterate teacher leaves students with the unhappy choice between disowning their own world as a fantasy, or rejecting the world of science as a fantasy.

Despite widespread discussion of the above issues in science education literature and despite the discomfort felt by teachers when facing imprecise and even dangerous understandings of the scientific activity, they normally have few opportunities to reflect on strategies to debate such issues with students or even with their peers. It is not easy to find didactic materials that are designed to provide a basis for such reflection and are based on episodes of the history of chemistry.

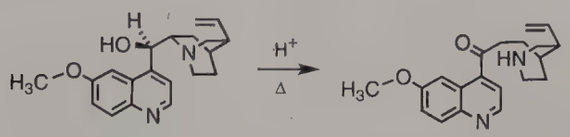
The aim of this article is to present a case study focused on the historical evolution of the quinine synthesis (an important medicine used in treatment of malaria) and designed to generate reflections about the process of the creation of scientific knowledge. It is appropriate for pre-service or in-service training of chemistry teachers or for graduate courses dealing with chemical knowledge in a broad sense. In this university, there is a graduate program in chemical education, whose participants are chemistry teachers and chemistry graduates. It offers disciplines of advanced contents in chemistry, general pedagogy, and science education. The case study presented in this article was developed to be discussed with students of this program, but can also be used in an advanced organic chemistry course. It offers an opportunity to discuss aspects of the nature of science by means of a concrete example that also demands the understanding of chemical concepts. It further allows the teaching of advanced organic chemistry topics, and the simultaneous discussion of the complex nature of scientific activity. The integrated approach presented here may serve as inspiration for the students in the program to develop their own case studies when teaching in high school or college level.

■ QUININE AS A SOUGHT-AFTER COMMODITY

Quinine is the main alkaloid extracted from the bark of trees belonging to the *Cinchona* genus, native to South America, of which there are around 40 different species. The molecular formula of quinine is $C_{20}H_{24}N_2O_2$, and its structure includes quinoline and quinuclidine rings. Three of the four asymmetric centers present in the molecule are located in the latter (Figure 1). The historical importance of quinine is directly related to its role in the treatment of malaria, a disease that has been identified throughout the globe (with the exception of polar and subpolar regions). According to Camargo (ref 12, p 212):

> Throughout the ages malaria has always been a great tormentor for mankind. Some epidemics, such as the plague of the 14th century, may have been more dramatic, due to the acute severity of their occurrence, but no other illness compares with malaria in the tenacious and perennial manner with which it afflicts mankind.

Scheme 1. Modern Representation of the Transformation of Quinine (Left) to Quinotoxine (Right), Undertaken by Pasteur in 1853



The extract of the cinchona bark was the first, and for a long time the only, truly effective medicine against malaria. The first descriptions of its use date from the 17th century, after which the medical literature contains countless reports of malaria cures using cinchona and around a thousand specific studies on the subject.[13] As its notoriety spread throughout the world, cinchona bark became a much sought-after commodity in colonial trade, motivating the French, Portuguese, and Spanish governments to organize expeditions in search of this wealth in various regions of the Americas. At the end of the 19th century, cinchona seeds were taken to the Dutch East Indies (now Indonesia), which in the next decades became the main supplier of cinchona to the West.

In 1820, Pierre Joseph Pelletier and Joseph Caventou isolated quinine from cinchona extract.[14] Identification of its structure, however, was only completed in 1907 by Paul Rabe,[15] a German chemist who dedicated several years of his scientific career to the investigation of *Cinchona* alkaloids, especially quinine.

Even prior to the proposal of its structural formula, several attempts had been made to synthesize quinine or at least to get a better understanding of its reactivity. In 1853, Louis Pasteur produced quinotoxine (at the time termed quinicine) by isomerization of quinine (Scheme 1). It is curious to observe that it was during an attempt to produce quinine, in 1856, that Perkin succeeded in the synthesis of mauveine, a landmark in the development of the chemical industry in the late 19th century.[16]

■ CASE STUDY IN QUININE SYNTHESIS

First Steps

In 1918, Paul Rabe and his German colleague Karl Kinder announced the first partial synthesis of quinine, in the form of a short communication.[17] The synthetic route proposed by these researchers is shown in Scheme 2, in which modern structural representation is used. Essentially, what Rabe and Kindler proposed was the reconversion of quinotoxine to quinine, the inverse of the process proposed by Pasteur in 1853. According to Rabe and Kindler's method, the partial synthesis of quinine consisted of the bromination of quinotoxine, followed by cyclization in a basic medium and reduction with aluminum. As a result, Rabe and Kindler produced a mixture of four isomeric alcohols, from which they isolated, by crystallization, quinine at a yield of 12% and quinidine at a yield of 5.5%.[17] Characterization of these substances was performed by means of their empirical formulas, fusion points, and optical rotations. The latter property was especially important for the characterization of quinine, as quinine is the only levorotatory molecule of the four epimers obtained after the reduction step.

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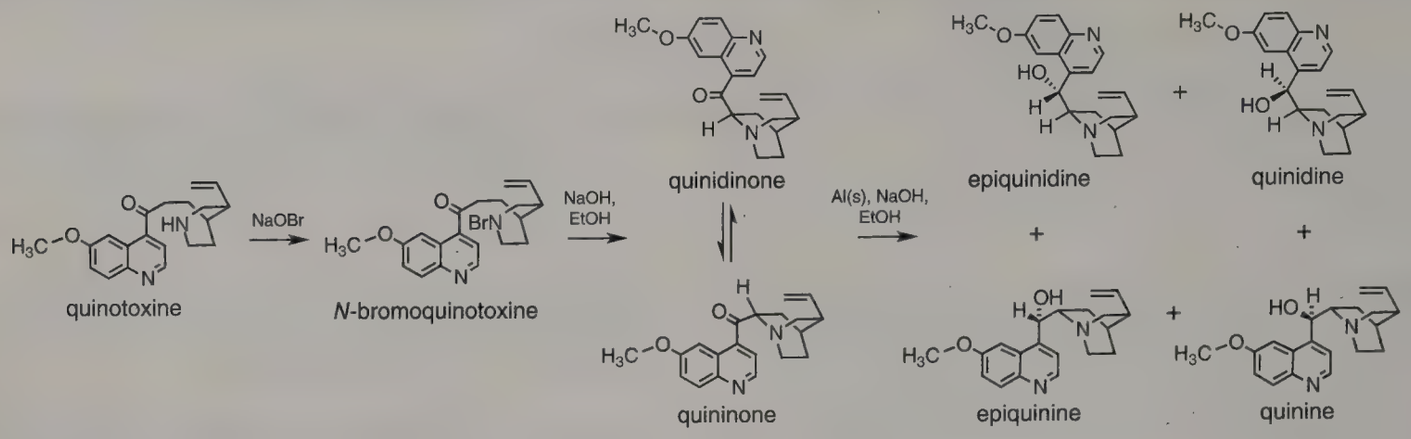
Scheme 2. Partial Synthesis of Quinine from Quinotoxine, As Proposed by Rabe and Kindler in 1918 (Modern Representation)



Despite the lack of experimental details provided by Rabe and Kindler,[17] Seeman[18] pointed out that there was evidence described by them, and by other authors, corroborating the claim for the partial synthesis of quinine, for the steps involving bromination and cyclization that were analogous to other reactions described earlier.[18] However, Rabe only provided further details about the reduction procedure with aluminum in 1932 and referred to a similar reaction, not to the synthesis of quinine itself.[18,19]

Total (Formal) Synthesis of Quinine

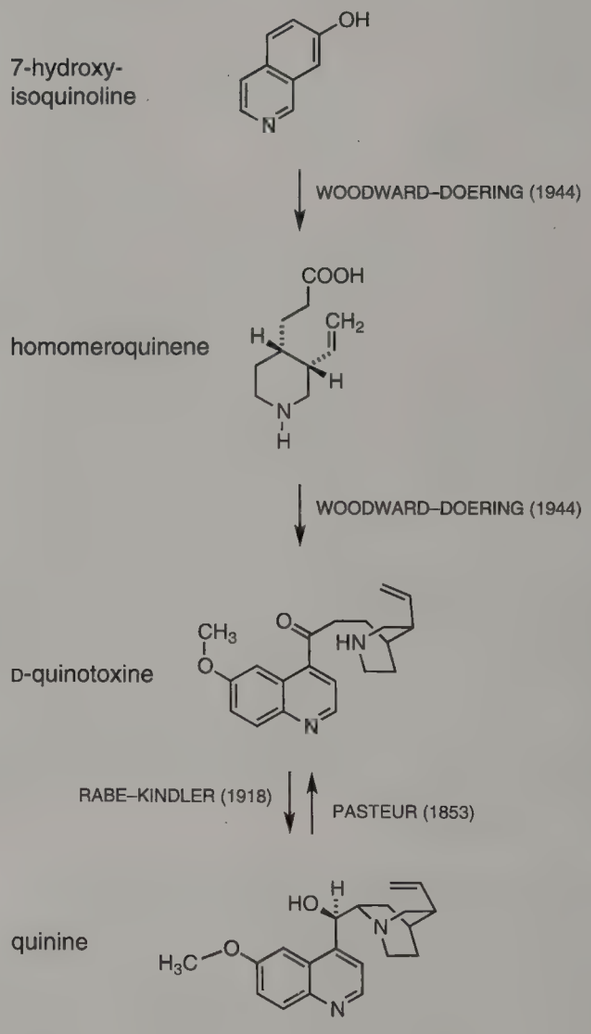
Despite the persistent lack of any detailed experimental description of the synthesis developed in 1918, Rabe and Kindler (especially the former) became the intellectual reference in matters concerning studies of the *Cinchona* alkaloids for the next decades. However, industrial production of quinine was still not feasible, because only a partial synthesis had been described, whose precursor (quinotoxine) was a substance that could only be obtained from quinine itself.

Meanwhile, malaria continued to claim victims and remained a concern to health agencies. In the 1930s, the global demand for quinine has been estimated as 7.5 times larger than the amount actually produced.[20] With the invasion of the Dutch East Indies by the Japanese during the Second World War, the Allies had their quinine supply cut, just when the drug was of critical importance.

Medicine was not the only area of application for quinine. Polaroid Corporation, a multinational company founded in 1937, was also interested in the substance because it was a chemical used in the manufacture of polarizing material. In 1942, two Harvard students, Robert Burns Woodward and William von Eggers Doering, were hired by Polaroid to investigate new polarizing materials, light absorbents, and optical plastics.[18] The most memorable result of this partnership was the announcement of the much-sought total synthesis of quinine in May 1944.[21] More extensive experimental details were only provided a year later, in a article entitled "*The Total Synthesis of Quinine*".[22] This news was received with worldwide enthusiasm, especially in the United States, where the press and the public heralded the researchers as war heroes because the possibility of manufacturing this vital medicine was seen as a strategic victory.[18]

The synthesis proposed by Woodward and Doering was based on the assumption that quinotoxine, the precursor to quinine in Rabe and Kindler's synthesis,[17] could be produced in laboratory. Prŏstenik and Prelog[23] proposed the synthesis of homomeroquinene

Scheme 3. Schematic Representation of the Total (Formal) Synthesis of Quinine As Proposed by Woodward and Doering in 1944 (Modern Representation)



(3-vinyl-4-piperidinepropionic acid) from cinchonine (of natural origin), and its conversion to quinotoxine by a process proposed by Rabe in 1919.[24] Woodward and Doering then proposed the total synthesis of quinine employing the synthesis of homomeroquinene from 7-hydroxy-isoquinoline, a substance that, apart from possessing almost all of the carbon and nitrogen backbone of homomeroquinene, had already been synthesized by Fritsch in 1895.[22] A graphical representation of the above-mentioned transformations is provided in Scheme 3.

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As shown in Scheme 3, one can see that the steps **actually** undertaken by Woodward and Doering in their laboratory were the conversion of 7-hydroxyisoquinoline to homomeroquinene, which was innovative and supported the claim that the total synthesis of quinine was achieved, and the obtainment of D-quinotoxine from homomeroquinene, reproducing what had already been proposed by Prŏstenik and Prelog in 1943. The final step, namely, the production of quinine, was not actually made by Woodward and Doering: after describing their experimental production of D-quinotoxine, the authors concluded, in their 1945 article (ref 22, p 868),

> In view of the established conversion of quinotoxine to quinine [reference to Rabe and Kindler's 1918 article], with the synthesis of quinotoxine the total synthesis of quinine was complete.

This assumption, which was in the basis of the authors' claim, was not questioned or criticized by the academic community, except for Gilbert Stork, then a graduate student at the University of Wisconsin–Madison.

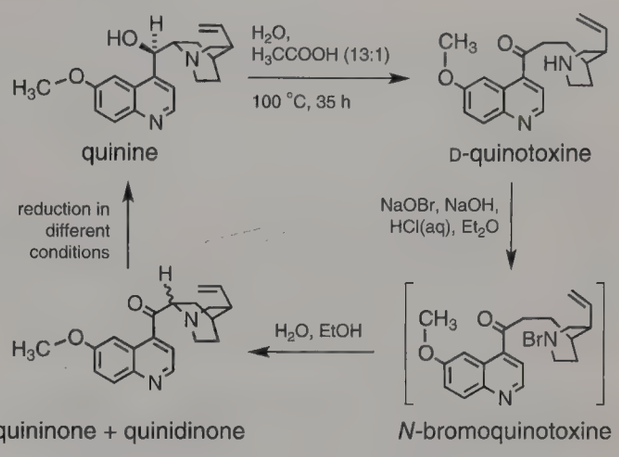
Gilbert Stork's Criticism

The interest of Gilbert Stork, born in 1921, in the synthesis of quinine began around 1940, as a result of his collaboration with Samuel M. McElvain, a graduate student at the University of Wisconsin–Madison, in a article published in 1946.[18] At the time of Woodward and Doering's publication, Stork's criticisms mainly concerned the fact that quinine had not (in any concrete sense) been produced at Harvard and the low yields achieved both in the steps proposed in 1918 and in those undertaken in 1945. With further developments in the field of stereochemistry from the 1940s onward, the lack of control over formation of the different quinine isomers (the four asymmetric centers in the structure allow the existence of 16 isomers) became another important focus of Stork's reservations on the attribution of the total synthesis of quinine to Woodward and Doering. Although the "cis" positioning of the vinyl and propionic acid groups on carbons 3 and 4 of the homomeroquinene piperidine ring has been established in 1944, Woodward and Doering's synthesis was not stereoselective and produced the homomeroquinene precursors as a mixture of "cis" and "trans" isomers in a proportion of almost 1:1.[22]

In 2001, 55 years after Woodward and Doering's synthesis, Stork published a article entitled "*The First Stereoselective Total Synthesis of Quinine*".[25] In this article, Stork and co-workers proposed an entirely stereoselective synthesis of quinine, infusing the text with historical considerations that left no doubt as to the position of the authors toward the synthesis announced in 1944. In the extract below, Stork et al.[25] argue that

> Woodward and Doering did not claim to have confirmed Rabe's 1918 report, in a few lines, that he had succeeded in converting quinotoxine to quinine (although the basis of their characterization of Rabe's claim as "established" is unclear), nor is there any evidence that they produced any quinine in their own laboratories. But this was wartime, and the U.S. had been cut off from the Dutch East Indies, its major source of cinchona bark. The resulting anxiety may explain press accounts, notable for enthusiasm rather than for sober analysis, which created the quasiuniversal impression that the construction of homomeroquinene in 1944 meant that quinine had been synthesized.

Scheme 4. Smith and Williams' (2008) Procedure for Conversion of D-Quinotoxine to Quinine



The 2001 publication and Stork's emphatic resistance to the notion that the first synthesis of quinine should be attributed to the Harvard researchers relegated the 1944 synthesis to the level of "myth" for many chemists.[26]

Despite his criticisms toward Rabe and Kindler's lack of experimental detail, and Woodward and Doering's alleged "excessive confidence", Stork himself did not try to reproduce the 1918 procedure.[18] However, this situation changed in 2008, with the work by Smith and Williams, from Colorado State University, who revisited Rabe and Kindler's controversial synthesis.

Rabe and Kindler Revisited

Smith and Williams[27] were studying a novel approach to the synthesis of quinine and other *Cinchona* alkaloids, based on intramolecular S_N2 reactions on C3 or C4. Motivated by the controversy surrounding the synthesis of quinine, and by Doering's 90th birthday, they decided to revisit the semisynthesis proposed in 1918, from a 21st century perspective. The very title of the resulting article ("*Rabe Rest in Peace: Confirmation of the Rabe–Kindler Conversion of D-Quinotoxine to Quinine*") already confirmed the successful reproduction of the 1918 procedure, and the text provided very interesting information on the subject. The procedure developed by Smith and Williams[27] is illustrated in Scheme 4.

However, Smith and Williams were surprised when nuclear magnetic resonance analysis, used to identify and quantify the products obtained, revealed the presence of only trace quantities of quinine, very different from the 12% yield reported by Rabe and Kindler in 1918. Intrigued not only by the very low yield of the reaction, but also by the fact that isolation of such small quantity of quinine would require techniques that did not exist in either 1918 or 1944, Smith and Williams investigated several different systems for reduction of the quininone/quinidinone mixture to quinine.

Inspection of the yields obtained using the different reducing systems suggests that the presence of Al^{3+} ions was responsible for a significant increase in the yield of quinine. The extent to which this fact affected Rabe and Kindler's observations is impossible to determine, because it is now impossible to discover either the origin of the aluminum used by them or the degree of purity of the reagent employed in their work.

Smith and Williams faced a number of difficulties in the development of their 2008 work that are worth mentioning. In

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contrast to Rabe and Kindler's description, *N*-bromoquinotoxine was somewhat unstable, for which reason it was promptly used in the subsequent step without prior purification (by crystallization, according to Rabe and Kindler). Crystallization was also found not to be efficient in separating the quininone/quinidinone mixture. Furthermore, the authors stated that significant quantities of quinine were only obtainable when the reduction step was performed with "aged" aluminum powder, that is, samples containing Al^{3+} surface impurities.[27]

Despite these difficulties, the authors claimed to have validated not only the Rabe–Kindler semisynthesis, but also the Woodward–Doering total formal synthesis, hence apparently closing, according to Philip Ball,[28] "a chapter in this fascinating history".

■ THE HISTORY OF THE SYNTHESIS OF QUININE AS A CASE STUDY FOR EDUCATORS IN CHEMISTRY

The case study presented here offers opportunities for reflection on several questions concerning the scientific enterprise, from its intellectual to sociological aspects. These questions may be particularly pertinent if presented during collective discussions on the nature of science in classroom situations. This section proposes some ideas and reflections that could provide useful starting points in debates concerning not only the history but the philosophy of science.

Every historical episode must be read and understood in its context. Hence, the work of Rabe and Kindler, Woodward and Doering, and finally Stork reflect not only different "states of the art" of synthetic chemistry, but were also surrounded by different social, economic, and political events. Therefore, to judge Rabe and Kindler for a lack of stereochemical specificity in their semisynthesis of quinine, based on information that only emerged more than 20 years later, would not be justifiable. Scientific knowledge is, therefore, **historical** and must be assessed within the context in which is elaborated.

The criticism arising from the lack of experimental detail in the Rabe and Kindler publication in 1918 paved the way for discussion of another interesting point: the announcement and authority of scientific knowledge. The article submitted for publication by the German researchers in 1918 was certainly assessed by their peers, was considered relevant to the point that should be shared with the international academic community, and eventually served as a reference for other works in the area. The same process undoubtedly occurred with Woodward and Doering. The peer review policy and the publication itself emphasize the **social** nature of scientific production, evidenced by the exchange of information via scientific journals. Conferences and meetings, and the personal interactions enabled by them, can be also included as means of social construction of the scientific knowledge.

Another important point about the construction and announcement of science contents can be exemplified, in this episode, by Stork and his criticism about the failure of Woodward and Doering in reproducing Rabe and Kindler's procedure. Although the intention here is not of adopting any definitive position, a reflection concerning the **legitimization of knowledge** would be relevant in this case. How can one define what is, or is not, validated knowledge or what can or cannot be referenced? Woodward and Doering were heralded as "national heroes" after the announcement of their work in 1944, which was then used as war propaganda. However, after Stork's publication in 2001, the achievement of Woodward and Doering was charged of being a "myth". In another twist, the reputations of Rabe, Kindler, Woodward, and Doering were saved by scientists who stepped forward to defend them, in articles published by Seeman,[18] and Smith and Williams.[27] Seeman analyzed original documents to conclude that none of the four authors could be accused of bad science, poor judgment, fraud, or incompetence. Smith and Williams returned to the laboratory to repeat Rabe and Kindler's experiments, testifying that their reported results were sound. The case study clearly shows that the concept of legitimacy is highly subjective and specific to an era and society and to a culture and a place. In the context of the scientific community, the journals are of particular relevance in this case and constitute, according to Lyman,[29] the main infrastructure of the scientific community (to obtain further information about the legitimacy of the scientific knowledge and its social aspects see, for example, refs 30–34).

The context of the publication of the total (formal) synthesis of quinine in 1944 also provides ample opportunity for reflection on the **non-neutrality** of science. As previously mentioned, quinine was a substance of utmost commercial and political interest during wartime. Therefore, the proposal of a method for its synthesis signified much more than the possibility for treatment of soldiers. Although the economic interests of Polaroid Corporation seem extra-scientific, a partnership between private capital and academic research undoubtedly contributed to an advancement of knowledge in the area of organic synthesis. Scientists can never achieve a completely unbiased approach of data. There are social trends and pressures that influence, and even determine, the scientific activity. During war times, such pressures may be especially powerful.

Finally, the **flexibility** of the scientific enterprise must be mentioned: the search for solutions to a specific problem can lead to unexpected knowledge. An excellent example is the synthesis of mauveine, proposed by Perkin in 1856, when he was actually trying to synthesize quinine. The case study may be used as an counterexample to the rigid and distorted view of science as an activity based on a scientific method to be followed mechanically, with no room for creativity, invention, or doubt, as was pointed out by Fernandez and colleagues (2002).[35]

An extract from Andriolli[36] summarizes and puts into perspective the observations on this topic

> Science is not isolated from the world, and social phenomena cannot be explained by natural laws (...) Even if there is no direct logical relationship between fact and value, there is a sociological relationship between the two, since knowledge of a fact leads to moral and political positions, and these values are present to the researcher, at all times, during the scientific process (...) As there is no absolute criterion to measure the scientific validity of knowledge, it is through critical publicity, in the clashing of ideas, that the results of research can be assessed, keeping in sight its correspondence with reality. But, even though a piece of scientific knowledge may have been accepted, it must remain under the condition of being refuted the moment that another understanding of reality might surpass it. It is not, therefore, a synonym of truth or a dogma, but the provisional result of a human investigation during a determinate historical and social period, and is, therefore, susceptible to all the ideas and values present in society.

■ FINAL REMARKS

Matthews[37] argues that a "sea of lack of meaning" had invaded the teaching of the sciences. Trying to repel this invasion, we agree with Matthews' assertion that historical and sociological

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studies can show that personal, cultural, and political interests of societies are involved in the examination of natural sciences, making science lessons more challenging and reflexive.[37] From this perspective, we propose the use of case studies, not only as a teaching strategy, but also as a tool to understand the complexity of scientific activity. Despite their importance and potential, case studies have been little used in classrooms and in investigative applications, and therefore deserve greater attention, not only by researchers concerned with the history of science and education, but also by those responsible for the task of mediation between science and the students, namely, teachers and prospective teachers.

■ ASSOCIATED CONTENT

Supporting Information

An extended version of the case study on the history of the quinine synthesis. This material is available via the Internet at http://pubs.acs.org.

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ARTICLE

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Prediction of log *P*: ALOGPS Application in Medicinal Chemistry Education

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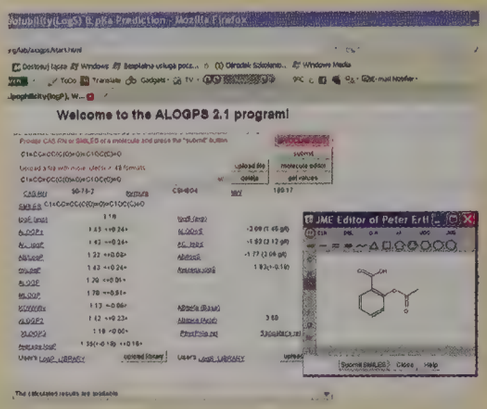
Supporting Information

ABSTRACT: Molecular hydrophobicity (lipophilicity), usually quantified as log *P* where *P* is the partition coefficient, is an important molecular characteristic in medicinal chemistry and drug design. The log *P* coefficient is one of the principal parameters for the estimation of lipophilicity of chemical compounds and pharmacokinetic properties. The understanding of log *P* parameter in the undergraduate medicinal chemistry course seems to be a pitfall for students. This parameter has typically been measured using experimental methods, but recently, log *P* has been determined using computational methods. The number of publications about lipophilicity predictions has gradually increased over the last 10 years, but the number of programs available for an online prediction of this important parameter remains limited. An interesting tool for calculation of log *P* coefficients is presented: the Virtual Computational Chemistry Laboratory (VCCLAB) package. The package includes the ALOGPS 2.1 program suitable for log *P* calculations. This software is accessible online and may be easily mastered by the undergraduate medicinal chemistry student.



KEYWORDS: Second-Year Undergraduate, Chemoinformatics, Organic Chemistry, Internet/Web-Based Learning, Bioanalytical Chemistry, Drugs/Pharmaceuticals, Laboratory Computing/Interfacing

During transport to the receptor, a drug usually passes through lipid membranes. Thus, the relative drug distribution between aqueous and nonpolar media is of considerable interest. The molecular hydrophobicity (lipophilicity) is normally quantified as log *P* where *P* is the partition coefficient obtained by measuring the drug distribution between two immiscible solvents, usually 1-octanol and water because 1-octanol properties are similar to those of natural membranes.[1] The octanol/water coefficient, *P*, is the ratio of a neutral molecule concentration in 1-octanol to its concentration in water when the phases are at equilibrium. The obtained values are consistent for nonionizable compounds. For charged substances that have greater water solubility than can be predicted from the neutral structure, often the term log *D* is used to describe the lipophilicity. The distribution coefficient, *D*, is calculated for the partition of a drug between 1-octanol and aqueous buffer. Both the partition and distribution coefficients are measures of how hydrophilic (water loving) or hydrophobic (water fearing) a chemical substance is. The hydrophobic drugs with high partition coefficients are preferentially distributed to hydrophobic compartments such as lipid bilayers of cells, whereas hydrophilic drugs of low partition coefficients are preferentially localized in hydrophilic compartments such as blood serum. The optimal lipophilicity range along with low molar mass and low polar surface area is the driving force that leads to good absorption of chemicals in the intestine by passive diffusion. That is why the log *P* coefficient is one of the principal parameters that estimates lipophilicity of chemical compounds and, to a large degree, indicates the pharmacokinetic properties. It is also used as one of the standard properties identified by Lipinski in the "rule of 5" for drug-like molecules.[2] It can be measured using known experimental methods,[3−6] but recently, computational chemistry (in silico) methods have widely been applied. The first method of log *P* calculation was developed by Hansch, Fujita, and Iwana.[6] Despite the incredible growth of the Internet, the number of practical online applications in drug design remains limited, particularly for predictions of drug-like compounds. For example, the number of methodological publications about lipophilicity predictions has gradually increased over the last 10 years, but the number of programs available for online prediction of this important property includes few applications.[7] Methods for log *P* calculation can be divided roughly into two major classes: the substructure-based methods, and the whole-molecule approaches.[8]

If a molecule contains basic or acidic groups, it becomes ionized and its distribution in octanol/water is pH dependent. At physiological pH, many basic or acidic drugs are ionized, and the partition coefficient is the distribution coefficient, *D*, which is generally accepted as the distribution between an aqueous buffer at pH 7.4 and 1-octanol. This distribution coefficient for monoprotic bases is defined as $\log D_{oct} = \log P_{oct} + \log [1/(1 + 10^{pKa - pH})]$. For

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Table 1. Examples of Some Web Resources in Chemoinformatics

No.	Name[a]	Provides	Link[b]
1	Corina	2D to 3D conversion of molecular structures	http://www.molecular-networks.com/software/corina/
2	Osiris	log *P*, solubility, toxicity, drug likeness	http://www.organicchemistry.org/prog/peo
3	VCCLAB	molecular descriptors, physicochemical properties	http://www.vcclab.org
4	Pre-ADMET	molecular descriptors and various ADME/T properties	http://preadme.bmdrc.org/preadme

[a] Information is adapted from ref 7. [b] All URLs accessed Sep 2011.

monoprotic acids, the equation has the same form, except that the exponent is written as "pH − pK_a". For polyprotic compounds, the equation becomes more complicated and is modified accordingly to incorporate correction terms for all of the ionized forms. Thus, log *D* prediction potentially accumulates errors due to the log *P* and pK_a predictions.[1] It is valuable to study log *P* to predict recognition and interactions between biological molecules because (i) log *P* is essentially an experimentally reproducible measurement; (ii) the partition experiments are inexpensive and can be performed relatively rapidly; and (iii) log *P* is directly related to the free energy of binding and solvation−desolvation effects.

■ DISCUSSION

The log *P* coefficient is a measure of lipophilicity, and many pharmaceutical and biological events are dependent on their lipophilic characteristics. Web resources are presented that can be applied by undergraduates for calculating log *P* coefficients for many organic derivatives. These tools can be helpful for rationalized drug design as the key step in medicinal chemistry education. From the examples of free computational Web resources in chemistry connected with this topic (Table 1), a user-friendly student package named Virtual Computational Chemistry Laboratory (VCCLAB), with emphasis on the ALOGPS 2.1 program,[9] is highlighted. The Web site of VCCLAB provides free online chemoinformatics tools, including the building and visualization of chemical structures, the calculation of molecular properties, and the analysis of relationships between chemical structure and properties. The ALOGPS 2.1 software provides an interactive online prediction of log *P*, water solubility, and pK_a of compounds for drug design and environmental chemistry studies. The software may be used in the classroom or laboratory and also in the dormitory, home, or local campus computer lab. Thus, it is a good supplementary reference for the undergraduate chemistry course and provides a new teaching method for the medicinal chemistry course.

The ALOGPS 2.1 program is built on the Associative Neural Network (ASNN) pattern, as a new challenge for the development of physicochemical data prediction methods.[10,11] In addition, in the parametrization of solubility prediction tools, some databases can be used, for example, PHYSPROP or AQUASOL.[12−15] To perform the log *P* calculations, the ALOGPS user can draw the molecule using the JME applet[16] or submit it in a format supported by freely available software such as OpenBabel.[17] A non-Java interface in the ALOGPS 2.1 package for structure submission is available as well. Moreover, the application calculates and compares lipophilicity and aqueous solubility of molecules using several methods including those described in references[16,18−21] (see Supporting Information), which definitely increases ALOGPS's practical viability. The prediction ability using ALOGPS 2.1 can also be increased in the library mode.[22]

■ HOW DOES IT WORK? AN EXAMPLE

To submit a compound into ALOGPS 2.1 program, the user needs to provide the compound SMILES code or enter its CAS registry number. These codes can be stored in databases, such as iResearch library[23] or ZINC.[24] To generate the SMILES code of a molecule, the student can use, for instance, the popular ACD/ChemSketch application.[25] For ionizable substances such as acetylsalicylic acid (aspirin, ASA, pK_a = 3.48), a well- known anti-inflammatory drug that can be easily synthesized by students,[26,27] the log *P* value is valid only at pH < 3; otherwise, the salt of acetylsalicylic acid becomes hydrophilic. Therefore, the log *D* parameter should be used for full characteristic of hydrophilic−hydrophobic properties of ASA. Because the problem of predicting log *D* is more complicated and it is computed from log *P* and pK_a, only the log *P* coefficient of ASA will be discussed here. After submission of the resulted SMILES code, the data from ALOGPS can be compared with that from other calculation methods, for example, ALOGPS = 1.43, MiLogP = 1.43, KOWWIN = 1.13, XLOGP2 = 1.42, XOLOGP3 = 1.19 (log *P* experimental = 1.19).[9] These differences are due to the calculation methods used in each of the different programs. The ALOGPS program, which is based on topological descriptors,[1,28−31] has been used in a series of studies on a variety of molecules, including libraries of drugs.[28,29] Although the program was developed to predict the partition coefficient log *P* for neutral compounds, it is user-friendly because it works in a completely automated fashion and does not require any user intervention or extended knowledge in computational chemistry.[28]

To assess the efficacy of ALOGPS, the program was made available to students attending the organic chemistry course for the second-year pharmacy students. The details of the student activity and assessment are described. In the first graded exercise, the students were given several structures taken from the PHYSPROP database;[13] two compounds were obtained by our scientific group[32,33] in a multistep synthesis. Based on the compounds, students were supposed to answer the yes−no question about the given calculated log *P* values (see Table 2 in the Supporting Information). To do that, the SMILES notification of 13 substances was required and was generated using ACD/ChemSketch application[25] according to the procedure discussed above. The results of the first graded exercise were 73% of the students passed and 27% of the students failed (N = 24). In the second exercise, the students were asked about the influence of the R group (Figure 1) on the log *P* coefficient. The question was which of the resulting derivatives became more hydrophilic or lipophilic in comparison with the reference compound, R = H (see Table 3 in the Supporting Information). That exercise examined a possibility of modification of pyrazole ring as the part of strategy in the organic synthesis of more hydrophilic or lipophilic analogues. The results of the second exercise were 98% positive answers. That exercise and the ALOGPS package as an example of SAR (structure−activity relationship) in medicinal

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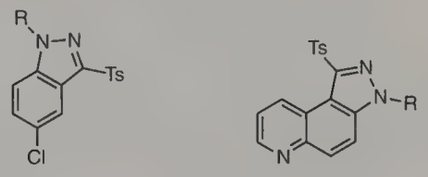

1 2

R = H, NO_2, CH_2NH_2, $(CH_2)_2O(CH_2)_2OH$, CH_2COOH, $(CH_2)_4CH_3$,

Ts = p-MeC_6H_4-SO_2

Figure 1. Structure of fused pyrazole derivatives used in the second exercise.

chemistry were then used to explain why some drugs containing electron-withdrawing substituents, such as the nitro group (e.g., nitro derivatives of benzodiazepines), were more lipophilic, able to diffuse over the blood–brain barrier, and to work longer in the human organism. It was also emphasized that oral drugs should have their lipophilicity between 1 and 4 on the log D scale to be absorbed by passive diffusion.[1] Because of this reasoning, the acetyl derivative of salicylic acid is used in oral treatment and is metabolized in the organism to the active salicylic acid.

■ PERSPECTIVES

Accurate prediction of log P is important for the pharmaceutical industry. Methods of log P prediction have attracted increasing interest during in the past decade. Given the benefits brought to bioinformatics by Web applications, it is attractive to encourage the development of these technologies in the chemoinformatics field. The Internet increases awareness about the existing software. The appearance of new protocols and standards for data sharing on the Web makes development of new applications easy and straightforward. The VCCLAB can be used as a prototype useful for developing such projects. The developed technology allows for integration of new third-party applications, which could be made available to the worldwide community. Fields such as chemistry and pharmacy benefit from having more chemists and pharmacists aware of Web software to reduce costly repetitions of work already done and to advance medicinal chemistry further and faster; VCCLAB, especially the ALOGPS 2.1 program, can be used as a springboard for research and development. With these tools becoming more accessible, students should be made aware of this information. Incorporating this program into instruction can enlarge the knowledge of undergraduate student on the chemoinformatics field and provide the medicinal chemistry instructor with interesting new material to incorporate across a range of classes. QSAR (quantitative structure–activity relationship) approaches such as ALOGPS, can improve prediction ability by self-learning on user-specific data and may also find significant application in the pharmaceutical industry in the near future.

■ ASSOCIATED CONTENT

Supporting Information

Materials used by students (handouts) and exercise examples. This material is available via the Internet at http://pubs.acs.org.

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Collinearity in Least-Squares Analysis

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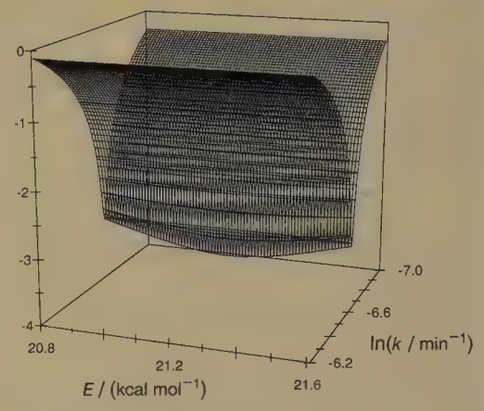
Supporting Information

ABSTRACT: How useful are the standard deviations per se, and how reliable are results derived from several least-squares coefficients and their associated standard deviations? When the output parameters obtained from a least-squares analysis are mutually independent, as is often assumed, they are reliable estimators of imprecision and so are the functions derived from them. But when these parameters have strong mutual dependencies, as is the rule rather than the exception in chemical data analysis, a more sophisticated approach to the statistical imprecision is required than is described in most chemistry textbooks. For the analysis of data from a typical kinetic experiment in physical chemistry, this is illustrated numerically as well as graphically.



KEYWORDS: First-Year Undergraduate/General, Second-Year Undergraduate, Analytical Chemistry, Physical Chemistry, Computer-Based Learning, Chemometrics, Kinetics, Molecular Mechanics/Dynamics, Quantitative Analysis, Reactions

The computer revolution has made many powerful numerical tools widely available, but chemical education has not always kept up by providing budding chemists with the necessary background information. A case in point is the common neglect of collinearity (and of its quantitative marker, the covariance) in the undergraduate training of chemists. The origin and precise meaning of the term "collinearity" are explained in some detail in the Appendix A (see the Supporting Information).

Insofar as undergraduate chemistry texts explain least-squares (or "regression") analysis, they usually assume that the sought parameters (such as the intercept and slope of a line fitted through the data) are mutually independent. This may be a conveniently simplification, to use as a starting point when subsequently followed by a more complete description, but leaving the topic at this level is misleading in the sense that, in many if not most chemical applications of least-squares analysis, mutually independent output parameters are the *exception* rather than the rule.

The most commonly used chemical application of least squares is in fitting experimental data to a straight line,

$$y = a_0 + a_1 x \qquad (1)$$

where y is the noise-carrying, dependent, or response *variable*; x the supposedly noise-free, independent, or control *variable*; and the adjustable, explanatory *parameters* are the intercept a_0 and the slope a_1. These adjustable parameters are often denoted as b and m, respectively, but here we will use the subscripted notation because it is readily extendable to polynomial expressions, such as quadratic or cubic models, as well as (with double indexing) to multivariate least squares, all models that are typically handled by a single computer program based on matrix algebra.

How does one distinguish between the independent and dependent variable? If the experimental uncertainty is exclusively (or, for practical purposes, predominantly) concentrated in one of the two variables, the variable with the largest relative uncertainty can be considered the dependent one. If one cannot make a clear distinction on this basis, a more sophisticated "general" least-squares routine may be needed instead.

In chemistry, the distinction between dependent and independent variables is usually obvious. In spectrometry, the wavelength or wavenumber is almost always more precisely defined than the observed signal from absorption, emission, and so forth; in a calibration curve, the measured signal (such as spectrometric or chromatographic peak height or area) is usually much more uncertain than the weight-based concentrations against which they are plotted; in kinetic measurements, the measured quantity is almost always much less precise than the elapsed time. (A notable exception is a titration of a strong acid with a strong base, where one usually measures pH as a function of added volume of titrant. While determining that volume can introduce significant experimental error, the noise in the pH reading varies from very low in the buffer regions, to an often much higher level near an equivalence point. In this particular case, the choice of dependent parameter is both unclear and, most often, appears to be rather inconsequential.)

However, even if the variables x and y are mutually independent, as they usually are in chemistry, the resulting least-squares parameters a_i are typically *not* so. Why is this so? The argument is easiest to understand for the straight line, eq 1, in which case we can specify the *covariance* v_{01} between the two resulting parameters a_0 and a_1 to quantify their mutual dependence. The definition of the covariance is given in Appendix A, and its specific form for least-squares fitting to a straight line is derived in

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Appendix B (see the Supporting Information) in algebraic, non-matrix format.

Our model will often fit a mathematically "smooth" function (such as a straight line, a power law, or a numerical simulation) to observed data whose experimental noise will make them somewhat random. When such noise is exclusively or mostly concentrated in y_{exp}, the quality of the fit of the model to the observations can be estimated in terms of the (vertical) *residuals*, that is, the differences between the corresponding experimental and model quantities, $y_{i,exp} - y_{i,model}$. Standard least-squares analysis is based on minimizing SSR, the *s*um of *s*quares of those *r*esiduals. For a straight line we then find the usual, closed-form solutions for the coefficients a_0 and a_1,

$$a_0 = \frac{\sum_{i=1}^{N} x_i^2 \sum_{i=1}^{N} y_i - \sum_{i-1}^{N} x_i y_i \sum_{i=1}^{N} x_i}{D} \tag{2}$$

$$a_1 = \frac{N \sum_{i=1}^{N} x_i y_i - \sum_{i=1}^{N} x_i \sum_{i-1}^{N} y_i}{D} \tag{3}$$

where the common denominator D is

$$D = N \sum_{i=1}^{N} x_i^2 - \left(\sum_{i=1}^{N} x_i\right)^2 \tag{4}$$

The *variance* v_{ff} of the fit of the model function $a_0 + a_1 x_i$ to the experimental data set y_i is directly defined in terms of the residuals, namely, as that *s*um of *s*quares of these *r*esiduals SSR divided by $N - P$, where N denotes the number of data points i analyzed, and P the number of adjustable parameters used in the model. For a straight line, we have two adjustable parameters, a_0 and a_1, so that $P = 2$.

The rationale for using $N - P$, often called the number of *degrees of freedom*, is that we can fit the data set exactly, no matter how severe the experimental noise, when $N = P$. Consequently, uncertainties in the fit are statistically meaningful only insofar as the number of data points N exceeds P. For the straight line $y = a_0 + a_1 x$, we therefore have

$$v_{ff} = \frac{\sum_{i=1}^{N} (y_{i,exp} - y_{i,model})^2}{N - P} = \frac{\sum_{i=1}^{N} (y_i - a_0 - a_1 x_i)^2}{N - 2} \tag{5}$$

and, as derived in Appendix B (Supporting Information), the associated variances v_{00} and v_{11} are found as

$$v_{00} \equiv v_{ff} \sum_{k=1}^{N} \left(\frac{\partial a_0}{\partial y_k}\right)^2 = \frac{v_{ff} \sum x^2}{N \sum x^2 - (\sum x)^2} \tag{6}$$

$$v_{11} \equiv v_{ff} \sum_{k=1}^{N} \left(\frac{\partial a_1}{\partial y_k}\right)^2 = \frac{v_{ff} N}{N \sum x^2 - (\sum x)^2} \tag{7}$$

where $\equiv$ denotes a definition, and where we have simplified the notation by deleting the indices i from the summations in the right-most expressions.

Because the two parameters a_0 and a_1 are derived *from the very same data* set, they will in general be mutually dependent, even if the values of x_i and y_i are not. To specify such a mutual dependency, which is important for the propagation of uncertainty in any results derived from both a_0 and a_1, one therefore defines a *covariance* $v_{01} = v_{10}$ between a_0 and a_1, which for a straight line is given by

$$v_{01} = v_{10} \equiv v_{ff} \sum_{k=1}^{N} \left(\frac{\partial a_0}{\partial y_k}\right)\left(\frac{\partial a_1}{\partial y_k}\right) = \frac{-v_{ff} \sum x^2}{N \sum x^2 - (\sum x)^2}$$
$$= \frac{-v_{00} \sum x}{N} = \frac{-v_{11} \sum x}{\sum x^2} \tag{8}$$

as detailed in Appendix B (Supporting Information).

As can be seen most clearly in the two right-most expressions in eq 8, these covariances are only zero when $\sum x = 0$, that is, when the average x-value is zero. Unfortunately, the above chemical examples involve only positive values of x, be it a wavelength or wavenumber, a concentration, or a time, and therefore must have non-zero x-averages. That was the basis for the earlier statement that collinearity of the resulting parameters a_0 and a_1 is the rule rather than the exception in chemical data analysis, and this will show as non-zero covariances of those parameters.

The square root of the variance v_{ii} is the standard deviation s_i, which has the advantage over the variance that it has the same dimension as the parameter it typically accompanies: the dimensions of $s_f = (v_{ff})^{1/2}$, $s_0 = (v_{00})^{1/2}$, and $s_1 = (v_{11})^{1/2}$, are those of y, a_0, and a_1, respectively. Unlike the variance, the covariance can be either positive or negative. Consequently, its square root, which might otherwise be considered a "co-standard deviation", is not defined, and it is seldom listed in the output of least-squares programs. But as is implied by the definitions of v_{00}, v_{11}, and $v_{01} = v_{10}$ in eqs 6–8, the variance and covariance can be of the same order of magnitude, and there is therefore no a priori reason to use one and ignore the other. They are typically displayed together in a covariance matrix of dimension $P \times P$.

We can define a related, more readily interpretable quantity, by normalizing the covariance. This is done by dividing v_{ij} by the square root of the product of the corresponding variances v_{ii} and v_{jj}, in which case we obtain the dimensionless *linear correlation coefficient* $r_{ij} = v_{ij}/(v_{ii}v_{jj})^{1/2} = v_{ij}/s_i s_j$ between the least-squares parameters a_i and a_j, where again $r_{ji} = r_{ij}$. In the limit of infinitely many measurements, $r_{ij} = 0$ implies that the two parameters a_i and a_j are mutually independent, but when $r_{ij} \neq 0$ (in that same limit), they are not. In practice, where we deal with a finite number of observations, as long as $|r_{ij}|$ does not exceed 0.8, a_i and a_j are at most weakly interdependent, while the covariance must usually be taken into account for $|r_{ij}| \geq 0.9$. Total collinearity corresponds with $|r_{ij}| = 1$.

Confusingly, linear correlation coefficients are often used on the *input variables* x and y rather than on the resulting *output parameters* a_0 and a_1.[1] In chemistry, the causal relationship of y on x can usually be taken for granted, in which case the resulting value of R or R^2 on the input variables is comfortably reassuring but oftentimes misleadingly so.

There have been numerous papers in the chemical literature dealing with the covariance, including in this and other educational journals in chemistry and physics,[2−19] and the concept goes back all the way to the influential 1826 book by Gauss,[20] but in view of the lack of its discussion in many chemical textbooks (with a few happy exceptions, such as refs 21 and 22), it may be useful to refresh our collective memory here and to introduce some tools to visualize this material. That is the purpose of this article.

■ AN EXAMPLE

As our example we will use a small data set from Bruice and Schmir[23] of the rate of spontaneous hydrolysis of *p*-nitrophenol

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	A	B	C	D	E	F	G
1	*T*	*k*	ln *k*	1/*RT*	$(\ln k)_{calc}$	*R* =	*1.9859E-03*
2	K	min^{-1}		$kcal\ mol^{-1}$			
3						*E* =	*21.2100*
4	298	5.60E-04	-7.487573774	1.68978434	-7.48189227	k_{25} =	*5.7344E-04*
5	303	1.02E-03	-6.887952652	1.66190011	-6.89046638	CM	*6.78841E-06*
6	308	1.83E-03	-6.303439312	1.63492121	-6.31824263	st.dev.	*3.3299E-04*
7	312	2.78E-03	-5.885304351	1.61396068	-5.87366880	*SSR* =	*3.9312E-04*
8		*Coeff:*	*28.35851653*	*-21.2100491*			
9		*StDev:*	*0.405843058*	*0.245907694*		*LinEst:*	
10		*Sf:*	*0.014020035*	LS1		*-21.2100491*	*28.35851653*
11		*CM:*	*0.164708588*	*-0.099785042*		*0.2459077*	*0.405843058*
12			*-0.099785042*	*0.060470594*		9.99731E-01	*0.014020035*
13		*CC:*	1	*-0.999850815*		7.43942E+03	2
14			*-0.999850815*	1		1.46230E+00	0.000393123

cell:	*instruction:*	*copied to:*
C4 =	LN(B4)	C5:C7
D4 =	1/($F$1*A4)	D5:D7
G3 =	-D8	
G4 =	EXP(C8+D8 (G1*298.15))	
F10:G14 =	LINEST(C4:C7,D4:D7,1,1)	

Figure 1. An Excel spreadsheet illustrating the analysis. The experimental data are listed in cells A4:B7, their transformed values in C4:D7, the resulting least-squares parameters in C8:D8 and G10:F10 for LS1 and LinEst, respectively, the corresponding standard deviations in C9:D9 and G11:F11, and the standard deviation of the overall fit in C10 and G12. The covariance matrix CM is given by LS1 in C11:D12, and the corresponding matrix of linear correlation coefficients CC in C13:D14, and shows two (in reality red) bold-printed values of $r_{01} = r_{10}$ to alert the user. The cell instructions are listed below the figure.

acetate in a 28.5% (v/v) ethanol/water mixture with 5.4 mM phosphate buffer at pH 8.0. Bruice and Schmir determined the rate constants k from concentration−time studies at different absolute temperatures T. We will use the Arrhenius equation

$$k_T = k' \exp[-E/(RT)] \tag{9}$$

in its logarithmic form

$$\ln k = \ln k' - E/(RT) \tag{10}$$

where k' is a constant, while the gas constant R has the value $1.985877_5 \times 10^{-3}$ kcal mol^{-1} K^{-1}. By plotting ln k versus $1/(RT)$, we can expect to see a straight line with intercept ln k' and slope $-E$.

We will illustrate our results on an Excel spreadsheet, because it is the most widely distributed computer platform for numerical data analysis and a number of convenient, freely downloadable add-ins[24] can be used to illustrate our point. Because the data set of Bruice and Schmir[23] is very short, its entire spreadsheet analysis can be shown. Moreover, the spreadsheets are annotated to show all formulas used. In combination with the explanatory text, they therefore provide complete, explicit data analysis instructions for undergraduate use.

The data in A1:B7 of Figure 1 are those of Bruice and Schmir, and Figure 2 shows a plot of the resulting values of ln k in C4:C7 versus $1/RT$ in D4:D7. This plot suggests that these data indeed fit the Arrhenius relation. Two linear least-squares analyses of the data in C4:D7, using either the macro LS (with its output shown in B8:D14) from my MacroBundle[24] or the Excel function LinEst (with its results displayed in F10:G14) are also shown in Figure 1. The slope a_1 and its sample standard deviation are found in cells D8 and D9, respectively (and, likewise, in cells F10 and F11) as $E = 21.2_1 \pm 0.2_5$ kcal mol^{-1}. (Here we will use a small nonsignificant guard digit to reduce possible rounding errors in relations subsequently derived from them. This is, of course, unnecessary as long as the data remain within the spreadsheet, which carries

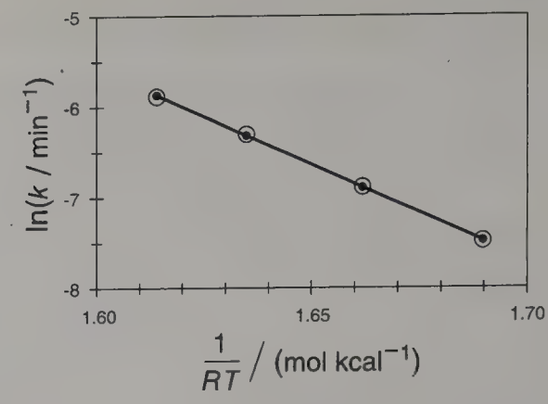

Figure 2. The natural logarithm of the experimental rate constants k versus $1/(RT)$ (open circles), where R is the gas constant and T the absolute temperature, for the hydrolysis of *p*-nitrophenol acetate according to Bruice and Schmir.[24] The line and small filled circles were calculated from the least-squares-fitted straight line through these points.

all numbers to 15 decimal places, regardless of their significance, as long as they are neither rounded nor truncated.)

Now consider deriving the value k_{25} of k at 25 °C from this fit. We repeat eq 10 as

$$\ln k_{25} = \ln k' - E/(RT_{25}) \tag{11}$$

where $T_{25} = 273.15 + 25 = 298.15$ K is the absolute temperature at our chosen reference temperature, 25 °C.

We can use eq 11 to calculate the sought rate constant k_{25} from the found parameters, $a_0 = 28.3_6 \pm 0.4_1$ for ln k', and $a_1 = -21.2_1 \pm 0.2_5$ for $-E$, as $k_{25} = \exp[a_0 + a_1/(0.0019858775 \times 298.15)] = (5._7 \pm 3._3) \times 10^{-4}$ min^{-1} when we use the usual propagation of uncertainty based on the standard deviations.

In Figure 2, we have used the macro Propagation[12,24] to calculate the standard deviation in k_{25}, but here is how you would do it if you had to do so manually. First, compute the standard deviation of ln k_{25} in eq 11 as $\{(s_0)^2 + [s_1/(RT_{25})]^2\}^{1/2} \approx [(0.4058)^2 + (0.2459/0.5921)^2]^{1/2} \approx 0.5807$. The (absolute) standard deviation $s_{k_{25}}$ in k_{25} is then the relative standard deviation $s_{\ln k_{25}}/\ln k_{25}$ in ln k_{25}, that is, $s_{k_{25}} = k_{25} \times s_{\ln k_{25}} \approx 5.734 \times 10^{-4} \times 0.5807 \approx 3.33 \times 10^{-4}$. It is a good thing computers exist to perform these tedious manual chores.

Note that the standard deviation of k_{25} so calculated in cell G6 is more than 50% of the value calculated in G4 for k_{25} itself, making this result seem highly uncertain, even though the original data were fitted to a straight line with an R^2 (the square of the correlation coefficient between the *input* data) of 0.99973, see cell F12 in Figure 1.

How can such a seemingly good fit as shown in Figure 2 yield such an imprecise result for k_{25}, especially when the experimental rate constants are given (and are therefore presumed good) to three digits, while one of them, at 298 K, lies quite close to the sought answer at 298.15 K? Where did we lose our precision?

The answer is not obvious when we use one of Excel's least-squares routines to analyze and plot data, because neither the least-squares function LinEst nor its related Regression macro displays the covariance v_{01} between a_0 and a_1, or the corresponding correlation coefficient r_{01}. Even worse, the outputs of neither TrendLine nor of the nonlinear least-squares routine Solver provide *any* uncertainty estimates of the coefficients a_0 and a_1 whatsoever. Many other least-squares routines are similarly deficient, possibly linked to the fact that the NIST test data sets

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for both linear and nonlinear least-squares[25] do not list any covariances either. But when you use software such as LS1 for fitting the experimental data, and Propagation for the propagation of uncertainty, you can observe the following.

Figure 1 shows the result for the standard deviation of k_{25} in G6 as obtained with Propagation, using the parameters in C8:D8 and their standard deviations in C9:D9. Identical results are obtained with the LinEst parameters in G10:F10 and the corresponding standard deviations in G11:F11. (The two sets are clearly quite similar, except that they show their results in opposite horizontal order. The more voluminous results of Excel's Regression macro, which uses LinEst as its computational engine, are no more informative in this respect.) This is also the result we earlier computed manually. But when we use Propagation with, as input, the *covariance matrix* in C11:D12 instead of the standard deviations in C8:D8, we obtain a different answer in cell G5, $6._8 \times 10^{-6}$, which is 50 times smaller than the value of $3._3 \times 10^{-4}$ found earlier in G6. The result obtained in G5 is certainly more consistent with the apparent uncertainty of the input rate constants.

The reason for the different results is that the correct expression for the variance of a quantity derived from two or more variables requires the explicit variances *and covariances* of the variables.[12,15] The all-too-common absence of covariances in the outputs of least-squares routines makes such a calculation impossible and, therefore, leads to incorrect conclusions. But when you fit the data with software that provides the covariances, such as LS1, and includes these covariances in the computation of error propagation (as described in ref 12 and implemented in the Propagation macro[24]), you can get correct uncertainty estimates even in the presence of strong collinearity.

An alternative approach would be to eliminate ln k' by subtracting eq 11 from eq 10,

$$\ln k = \ln k_{25} + \frac{E}{R}\left(\frac{1}{T_{25}} - \frac{1}{T}\right) \qquad (12)$$

and then to analyze ln k as a function of $(1/RT_{25} - 1/RT)$. If we do that, we find $E = 21.2_1 \pm 0.2_5$ kcal mol^{-1}, and ln $k_{25} = -7.46_4 \pm 0.01_2$ from which k_{25} follows as $(5.73_4 \pm 0.06_8) \times 10^{-4}$ min^{-1}, either directly or with the aid of the Propagation macro.

■ CENTERING

Which result for the standard deviation of k_{25} is correct, $s_{25} = 3._3 \times 10^{-4}$ min^{-1} (58% of k_{25}) or $s_{25} = 6._8 \times 10^{-6}$ min^{-1} (1% of k_{25})? For the answer, we turn to centering, which will make the covariances zero for a straight line, in which case both approaches should yield the same results. (Least-squares fitting to a polynomial requires another type of centering.[26]) To this end, we compute in cell G19 the average value $(1/RT)_{av}$ of $1/RT$, copy the data in A1:C7 to A18:C24, in D21:D24 compute $1/RT - (1/RT)_{av}$, modify its heading in D18 to reflect that change, and repeat the analysis, as shown in Figure 3. In other words, we analyze

$$\ln k = \ln k'' + E\left[\left(\frac{1}{RT}\right)_{av} - \frac{1}{RT}\right] \qquad (13)$$

where ln k = ln k'' when $1/T = (1/T)_{av}$, in our case at about 32.01 °C.

We now compute k_{25} in cell G21 as $\exp[a_0 + a_1\{1/RT_{25} - (1/RT)_{av}\}]$ and obtain the same value as before. There is, however,

	A	B	C	D	E	F	G
18	*T*	*k*	ln *k*	$(1/RT)_{av}$-1/*RT*	$(\ln k)_{calc}$	*R* =	*1.9859E-03*
19	K	min^{-1}		kcal mol^{-1}		$(1/RT)_{av}$ =	**1.65014159**
20						*E* =	*21.2100*
21	298	5.60E-04	-7.487573774	-0.03964275	-7.481892271	k_{25}=	*5.7344E-04*
22	303	1.02E-03	-6.887952652	-0.01175852	-6.890466384	CM	*6.78841E-06*
23	308	1.83E-03	-6.303439312	0.01522037	-6.318242635	st.dev.	*6.7884E-06*
24	312	2.78E-03	-5.885304351	0.03618090	-5.873668800	*SSR* =	*3.9312E-04*
25		*Coeff:*	*-6.641067522*	*21.2100491*			
26		*StDev:*	*0.007010017*	*0.245907694*		*LinEst:*	
27		*Sf:*	*0.014020035*	LS1		*21.2100491*	*-6.641067522*
28		*CM:*	*4.91403E-05*	*0*		*0.2459077*	*0.007010017*
29			*0*	*0.060470594*		9.99731E-01	0.014020035
30		*CC:*	1	*0.0000000*		7.43942E+03	2
31			*0.0000000*	1		1.46230E+00	0.000393123

cell:	*instruction:*	*copied to:*
C21 =	LN(B21)	C22:C24
D21 =	$G$19-D4	D22:D24
E21 =	$C$25+$D$25*D21	E22:E24
G19 =	AVERAGE(D4:D7)	
G20 =	D25	
G21 =	EXP(C25+D25*(G19-1/(G18*298.15))	
G24 =	SUMXMY2(C21:C24,E21:E24)	
F27:G31 =	LINEST(C21:C24,D21:D24,1,1)	

Figure 3. The same Excel spreadsheet as shown in Figure 1, extended to include a centered analysis of the same input data. For reasons to be discussed shortly, the spreadsheet also includes a column for $(\ln k)_{calc}$ and a cell for computing SSR.

a major change in its associated standard deviation, which is now *the same* whether we use either the standard deviations of a_0 and a_1, or their covariance matrix, because in this case the covariances v_{01} and v_{10} are zero, see cells C31 and D30. This common value for the standard deviation of k_{25} in cells G22 and G23 is equal to that in cell G5 of Figure 1, which therefore must have been the correct answer. Obviously, unless we center the data, or use eq 12 (in which case, we avoid propagating the uncertainty by formulating the least-squares problem directly in terms of the desired parameters, in this case E and ln k_{25}), we can still find the correct answer for the standard deviation of k_{25}, but *not* by using the standard deviations of a_0 and a_1 in computing the propagation of uncertainty. Instead, we then must use the covariance matrix. When the covariances are not provided, any subsequent propagation of imprecision risks being useless, misleading busywork.

On the other hand, when the covariance matrix is part of the output of a least-squares routine, and is combined with the Propagation macro, or similar software that utilizes the covariance in its calculations, we find the correct uncertainty estimates no matter which method we use. This makes use of the covariance matrix the more general approach, as well as the more convenient.

■ THE ERROR SURFACE

The least-squares method minimizes the value of SSR. To *visualize* how broad or narrow that minimum is, we turn to its error surface or, as it was called by Sillén et al.,[27] its pit map, that is, a map of log SSR as a function of the variables, here E and ln k. We use ScanF, a new addition to the MacroBundle,[24] to compute values of log SSR for various values of a_0 and a_1. We then call Mapper (in that same MacroBundle) for a more readily grasped color (or, as shown here, gray scale) rendition. Instead of Mapper we can use Excel's 3-D surface graph. Results for using Mapper0 for the regular and centered approach are shown in Figures 4 and 5, respectively.

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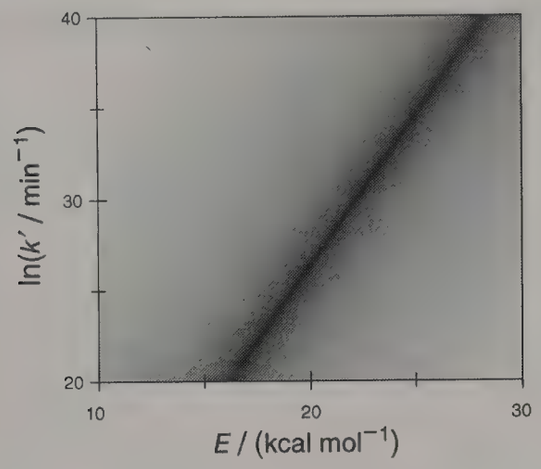

Figure 4. The error surface, a map of log SSR (where darker grays represent lower values), as a function of E (varying between 10 and 30, with steps of 0.1) and ln k' (in the range 20 (0.1) 40), for the noncentered approach of Figure 2.

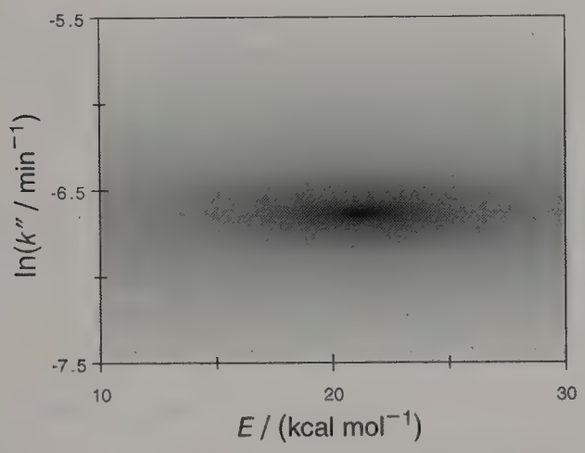

Figure 5. A similar map of log SSR for a centered least-squares analysis of ln k vs $1/RT - (1/RT)_{av}$, with E = 10 (0.1) 30 and log k'' = −7.5 (0.1) −5.5.

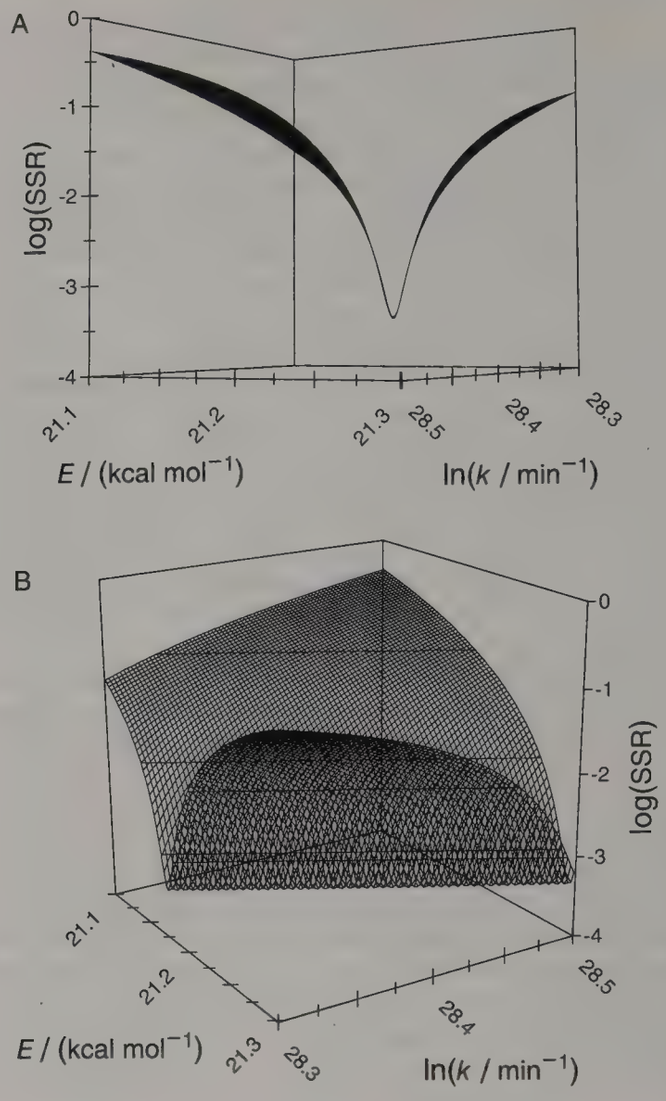

Figure 6. (A) A 3-D surface graph of log SSR for the noncentered least-squares analysis, looking inside and along the trench shown in Figure 4. (B) A view of the 3-D graph from a perpendicular angle, by pulling the right-bottom corner of Fig. 6A at E = 21.3 kcal mol^{-1}, ln k = 28.3, and log(SSR) = −4 to become the front-bottom corner in Fig. 6B. The minimum is too shallow to be visible in this side-view.

To use ScanF, place a horizontal scale of numbers, such as 10.0, 10.1, 10.2, ..., 29.9, 30.0 in cells I4:DE4 by depositing the number 10 in cell I4, the instruction =I4+0.1 in J4, and copying this all the way to cell DE4. Likewise, in H5 deposit the value to 40.0, in H6 the instruction =H5−0.1, and copy this down to H205. Leave cell H4 empty, unless you want to specify minimum and maximum scale values. Highlight the block H4:DE205, and call ScanF. After ScanF displays its results, call Mapper.

In the present case, such an error surface can illustrate an interesting aspect of collinearity, namely, that the mutual dependence between two parameters can distort the usual "pit" into a drawn-out shape that more resembles a trench of near-uniform depth, see Figure 4, indicating that the analysis is ill-conditioned. We can understand this as follows: when a data point has a pronounced error, the least-squares fit will respond by changing its best-fitting parameters. It will change more when the point is at one of the ends of the data range, when there are few points in the data set to act as counterbalance and when the deviation is extreme. When the minimum in log(SSR) is sharp, the data effectively resist this change. On the other hand, in the presence of pronounced collinearity, the other output parameter (there are only two in fitting to a straight line) can adjust to minimize the resulting change in SSR, and the minimum in the error surface becomes a drawn-out trench rather than a well-contained pit.

For the mathematically inclined, here is another explanation. When there is a strong linear correlation between a_0 and a_1, the corresponding matrices $\mathbf{X}$ and $\mathbf{X}^T$ in Appendix A (Supporting Information) are near-singular, that is, ill-conditioned. Inversion of the matrix product $\mathbf{X}\mathbf{X}^T$ by one of the usual Gauss elimination procedures will then *amplify* any noise in the matrix elements by a factor of the order of κ, the condition number of the matrix. The more ill-conditioned the matrix is, the larger the uncertainties in the resulting coefficients. In extreme cases, the values of those coefficients may be so corrupted by amplified noise so as to be completely wrong. It would therefore be incorrect to consider collinearity to affect only the standard deviations; the latter are merely the early warning signs, the canaries in the coalmine, indicating that something is wrong with the parameters themselves.

Figure 6 shows a 3-D graph of the region near the minimum in log SSR of the noncentered approach, presenting two views: one looking along the length of the trench, the other perpendicular to it. We see that the trench is deep and narrow in one direction, with an almost horizontal bottom, making it difficult to determine the

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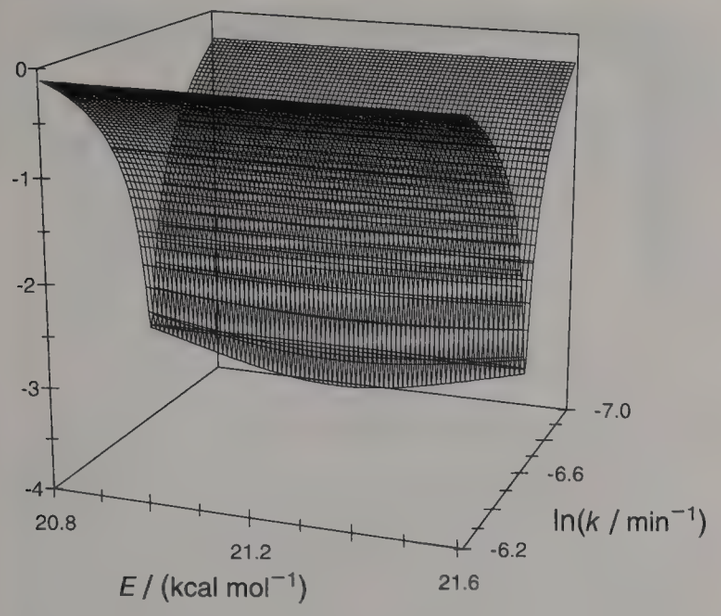

Figure 7. A 3-D image of the region around the minimum in log SSR for the centered approach. The minimum value of log SSR is now more visible than in Figure 6B, partially because E is shown over a wider range.

	A	B	C	D	E	F	G	H
1	*T*	*k*	ln *k*	*w*	1/*RT*	ln k_{calc}	*R* =	*1.9859E-03*
2	K	min^{-1}			kcal mol^{-1}			
3							*E* =	*20.8385*
4	298	5.60E-04	-7.487574	3.14E-07	1.68978434	-7.461195377	k_{25} =	*5.8525E-04*
5	303	1.02E-03	-6.887953	1.04E-06	1.66190011	-6.880131069	CM	*1.4420E-05*
6	308	1.83E-03	-6.303439	3.35E-06	1.63492121	-6.317932485	st.dev.	*5.1301E-04*
7	312	2.78E-03	-5.885304	7.73E-06	1.61396068	-5.881147431	*SSR* =	*9.8433E-04*
8			*Coeff:*	*27.75130171*	*-20.83845629*			
9			*StDev:*	*0.607884925*	*0.373937781*			
10			*Sf:*	0.013416588	WLS1			
11			*CM:*	*0.369524082*	*-0.227297298*			
12				*-0.227297298*	*0.139829464*			
13			*CC:*	1	*-0.999939107*			
14				*-0.999939107*	1			

cell:	*instruction:*	*copied to:*
C4 =	LN(B4)	C5:C7
D4 =	B4^2	D5:D7
E4 =	1/($H$1*A4)	E5:E7
F4 =	$D$8+$E$8*E4	F5:F7
H3 =	-E8	
H4 =	EXP(D8+E8/(H1*29815))	
H7 =	SUMXMY2(C4:C7,F4:F7)	

Figure 8. The spreadsheet for the weighted but not centered least-squares analysis of the data in cells A4:B7, with added parts (F4:F7,G7:H7) to facilitate mapping and displaying log SSR.

precise location of its minimum. The centered approach depicted in Figures 5 and 8 does not have that problem, and finding its minimum value for log SSR is therefore much better defined, because the less collinear system is more tolerant of noise.

The 2-D map of the error surface can provide a quick overview, while its 3-D surface visualization has the advantage that it can be rotated and tilted to present optimal viewing angles. The head-on view of the trench in Figure 6A is even better *in action* on your spreadsheet screen. While keeping down Ctrl, grab a corner of the box around the wire-frame, and move that corner ever so slightly to evoke a redraw. This will successively scan the individual curves, sequentially displaying the single profiles that make up the graph.

■ WEIGHTED LEAST SQUARES

There is an inherent problem with the above analysis, in that it minimizes the sum of squares of the residuals in ln k whereas the original data were k-values. This is readily remedied by using a

	A	B	C	D	E	F	G	H
18	*T*	*k*	ln *k*	*w*	$1/RT - w/(RTw)_{av}$	(ln *k*)$_{calc}$	*R* =	*1.9859E-03*
19	K	min^{-1}			mol kcal^{-1}		$w/(RTw)_{av}$ =	*1.62553221*
20							*E* =	*20.838456*
21	298	5.60E-04	-7.487574	3.14E-07	0.06425212	-7.46120	k_{25}=	*5.8525E-04*
22	303	1.02E-03	-6.887953	1.04E-06	0.03636789	-6.88013	CM	*1.4420E-05*
23	308	1.83E-03	-6.303439	3.35E-06	0.00938900	-6.31793	st.dev.	*1.4420E-05*
24	312	2.78E-03	-5.885304	7.73E-06	-0.01157153	-5.88115	*SSR* =	*9.8433E-04*
25			*Coeff:*	*-6.122280303*	*-20.83845629*			
26			*StDev:*	*0.006708296*	*0.373937906*		w_{av} =	3.10781E-06
27			*Sf:*	0.013416592	WLS1		w/w_{av}	w/RTw_{av}
28			*CM:*	*4.50012E-05*	*-2.30437E-18*		0.1009	0.170510363
29				*-2.30437E-18*	*0.139829557*		0.3348	0.556350783
30			*CC:*	1	-9.18631E-16		1.0776	1.761742583
31				-9.18631E-16	1		2.4868	4.013525130

cell:	*instruction:*	*copied to:*
C21 =	LN(B21)	C22:C24
D21 =	B21^2	D22:D24
H26 =	AVERAGE(I21:I24)	
G28 =	D21 $H$26	G29:G31
H28 =	G28/E4	H29:H31
H19 =	AVERAGE(E4:E7)	
E21 =	E4-$H$19	E22:E24
F21 =	$D$25+$E$25*E21	F22:F24
H20 =	-E25	
H21 =	EXP(D25+E25*(1/(H18*298.15)-H19))	
H24 =	SUMXMY2(C21:C24,F21:F24)	

Figure 9. The spreadsheet for the weighted, centered least-squares analysis of the data in cells A21:B24, with added parts (F18:F24,G24:H24) to facilitate mapping log SSR. The input data are in columns A and B, the data analysis in C:F, and the results in G:H.

weighted least-squares analysis. We can do so by inserting a column for the "global" weights w[28] between those for ln k and $-1/RT$, assign those weights w as equal to k^2 because

$$w \equiv \frac{1}{(\mathrm{d}\ln k/\mathrm{d}k)^2} = \frac{1}{(1/k)^2} = k^2 \qquad (14)$$

and by then using the macro WLS1 instead of LS1, see Figure 8.

We find $E = 20.8_4 \pm 0.3_7$ kcal mol^{-1} and $k_{25} = (5._9 \pm 5._1) \times 10^{-4}$ min^{-1} when we use $1/RT$ as our independent variable and the standard deviations to estimate their precision, and $k_{25} = (5.8_5 \pm 0.1_4) \times 10^{-4}$ min^{-1}, using the covariance matrix rather than just the standard deviations. In this case, using only the standard deviations of a_0 and a_1 would yield a value for the standard deviation of k_{25} that is a whopping 88% of the value of k_{25} itself!

The corresponding analysis with both weighting and centering, that is, by using as the independent variable $1/RT_i - w_i/RT_iw_{av}$, is shown in Figure 9 and yields $E = 20.8_4 \pm 0.3_7$ kcal mol^{-1} and $k_{25} = 5.8_5 \pm 0.1_4$ with either the covariance matrix or the standard deviations. (Note that WLS uses relative weighting, and that we therefore have used w_i/w_{av} times $1/RT_i$ in computing the amount of centering in cell H19.) Bruice and Schmir[23] reported E as 20.7 kcal mol^{-1}.

■ NONLINEAR LEAST SQUARES

The ready availability of nonlinear least-squares routines has made those an attractive alternative to linear least-squares. (In this context, the term "linear" does not define the model $y = f(x)$, which can be quite nonlinear, but instead implies that the expression $f(x)$ is a linear function of the model parameters a_i.) A main advantage of using nonlinear least-squares is that one need not have (or know of) a method to convert the data into a format that fits the requirements for linear least-squares, that is, as a power series, either directly or through some mathematical transformation. In the latter case, nonlinear least-squares avoids the need for global weighting, an approach that is not without its

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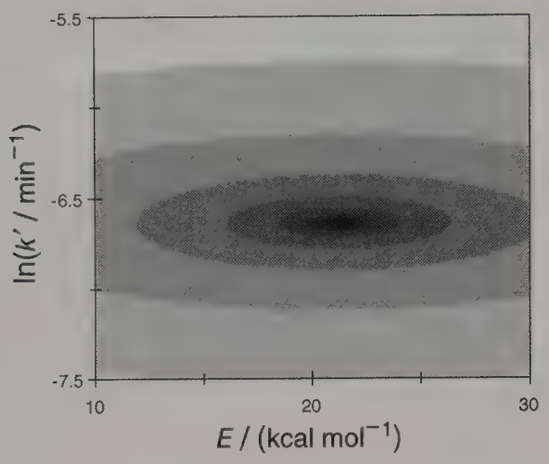

Figure 10. The error surface for the spreadsheet of Figure 9 made with Mapper00, which simulates a contour-like diagram by using a stepwise rather than a gradual gray scale. The same plot made with Mapper0 would look quite similar to that of Figure 5.

	A	B	C	D	E	F
1	*T*	*k*	k_{calc}	*R = 1.9859E-03*		
2	K	min^{-1}				
3						
4	298	5.60E-04	0.000099153	*SSR = 1.0349E-05*		
5	303	1.02E-03	0.000131041	*E = 10.0000*		
6	308	1.83E-03	0.000171623	k_{25} *= 1.0000E-04*		
7	312	2.78E-03	0.000211644			
8						
9						
10						

cell: instruction: *copied to:*
C4 = $E$6*EXP(($E$5/$E$1)*(1/298.15-1/A4)) C5:C7
E4 = SUMXMY2(B4:B7,C4:C7)

Figure 11. The spreadsheet ready for analyzing the Bruice and Schmir data by nonlinear least-squares.

own pitfalls, see (ref 26, section 4.15). Weighted least-squares typically fails in the presence of significant amounts of random noise, whereas nonlinear least-squares tends to be much more noise-resilient.

A main disadvantage of nonlinear least-squares is that, absent reasonably close initial parameter estimates, one can in principle miss the mark by getting hung up in a so-called false (that is, local rather than global) minimum. Here, mapping the error surfaces can provide a useful monitor of the absence of such false minima in the neighborhood scanned. Figures 4–7 and 10 indicate no such nearby false minima in our case.

On spreadsheets, the most common nonlinear least-squares routine available is Solver, a quite powerful general optimization program[29–31] that can deftly handle multiple variables, where necessary with user-imposed constraints. Unfortunately, the version of Solver supplied with spreadsheets does not provide any uncertainty estimates of its output parameters, but the macro SolverAid[24] can fill this gap in Excel.

How can we apply Solver in our example? We make columns for the experimental data T and k, compute a column for k_{calc}, which we calculate from assumed, plausible values for k_{25} and E using eq 12, and then compute SSR. Figure 11 illustrates a possible layout and the specific instructions used. When in doubt, subsequently use different initial values to see whether that changes the answer obtained by Solver; with reasonably nearby starting values it should not.

	A	B	C	D	E	F
1	*T*	*k*	k_{calc}	*R = 1.9859E-03*		
2	K	min^{-1}				
3				SolverAid		
4	298	5.60E-04	0.000574602	*SSR= 1.1152E-09*	*2.36131E-05*	
5	303	1.02E-03	0.001027578	*E= 20.846295*	*0.369240958*	
6	308	1.83E-03	0.001803293	k_{25} *= 5.8488E-04*	*1.4242E-05*	
7	312	2.78E-03	0.002791458	*CM:*	*0.136338885*	*-5.05589E-06*
8					*-5.05589E-06*	*2.02834E-10*
9				*CC:*	*1*	*-0.961430484*
10					*-0.961430484*	*1*

cell: instruction: *copied to:*
C4 = $E$6*EXP(($E$5/$E$1)*(1/29815-1/A4)) C5:C7
E4 = SUMXMY2(B4:B7,C4:C7)

Figure 12. The final spreadsheet after using Solver and SolverAid.

Call Solver, select Options, extend its Precision by adding six zeroes to make it 0.000000000001, and place tick marks in front of Use Automatic Scaling and Central Derivatives, these being the three general changes we typically make before applying Solver to a scientific problem. (Try for yourself what you get without using these options. They are useful general settings to start with, but not all three are always needed, or even optimal, for all types of chemical problems.) We then click OK, and in this particular case, Set Target Cell: E4 (the value of SSR), Equal To Min (because we want to minimize SSR), By Changing Cells: E5:E6 (the adjustable parameters E and k_{25}), press Solve, and then press OK to Keep Solver Solution. This yields the values for SSR, E, and k_{25} shown in cells E4:E6 of Figure 11, but no uncertainty estimates. We then rerun Solver (which is easy since it remembers our last settings) using the just-obtained results as our input data, because that sometimes refines the answer. If its answer does not change, or does so only in its last decimal, we have reached its limit.

For the uncertainty estimates call SolverAid from the Macro-Bundle,[24] give it the location of the column containing the Solver parameters as E5:E6, the Solver Target as E4, the column containing Y_{calc} as C4:C7, and press OK. Specify E7:F8 for the covariance matrix, and approve E9:F10 for the array of correlation coefficients. The final result will then look like Figure 12.

■ COMPARISON OF RESULTS

For ease of comparison, all numerical results obtained so far for the activation energy E and the standard rate constant k_{25} are listed in Table 1. Keep in mind that their standard deviations are undoubtedly overoptimistic estimates, because the input data were obtained from data fitting of k values determined by prior data analysis. However, they were used here without experimental uncertainty estimates, because none were reported. Moreover, standard deviations based on fitting just four data points with two parameters have themselves a relative standard deviation of fully 50% (see ref 26, section 2.12). All we can claim, therefore, is that the bold-faced results are consistent with the input data used.

From Table 1, we see that we here obtain fully equivalent results either with weighted linear least-squares (WLS1) using the covariance matrix, with centered weighted least-squares, or with nonlinear least-squares (Solver plus SolverAid), even though the latter method uses a quite different algorithm, so that its output can differ in statistically insignificant digits. The same cannot be said for the results obtained with LinEst or Regression, with which it is *impossible* to find the proper value and standard deviation for k_{25}, while TrendLine and Solver do not even try to provide uncertainty estimates.

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Table 1. The Least-Squares Results Obtained with the Small Data Set of Bruice And Schmir[23a]

Method	tool	E / (kcal mol^{-1})	$k_{25}/(10^{-4}$ min$^{-1})$ from st. dev.	$k_{25}/(10^{-4}$ min$^{-1})$ from CM
Simple linear least-squares	LS1	$21.2_1 \pm 0.2_5$	$5._7 \pm 3._3$	$5.73_4 \pm 0.06_8$
Simple linear LS using eq 12	LS1	$21.2_1 \pm 0.2_5$	$5.73_4 \pm 0.06_8$	
Centered linear least-squares	LS1	$21.2_1 \pm 0.2_5$	$5.73_4 \pm 0.06_8$	$5.73_4 \pm 0.06_8$
Weighted linear least-squares	WLS1	$\mathbf{20.8_4 \pm 0.3_7}$	$5._8 \pm 5._1$	$\mathbf{5.8_5 \pm 0.1_4}$
Centered and weighted linear LS	WLS1	$\mathbf{20.8_4 \pm 0.3_7}$	$\mathbf{5.8_5 \pm 0.1_1}$	$\mathbf{5.8_5 \pm 0.1_4}$
Nonlinear least-squares	Solver + SolverAid	$\mathbf{20.8_5 \pm 0.4_0}$	$\mathbf{5.8_5 \pm 0.1_4}$	

[a] The standard deviations were computed with the propagation macro of the Macrobundle.

Table 2. A Few Values for the Coordinates of the Trench in Figure [4] in the Neighborhood of the Minimum

a_0	a_1	SSR	log SSR
27.5168633	−20.7	0.00123875	−2.9070170
27.6818775	−20.8	0.00093967	−3.0270258
27.8468916	−20.9	0.00070560	−3.1514430
28.0119058	−21.0	0.00053654	−3.2703994
28.1769199	−21.1	0.00043249	−3.3640247
28.3419341	−21.2	0.00039345	−3.4051093
28.3585165	−21.2100491	0.00039312	−3.4054718
28.5069482	−21.3	0.00041942	−3.3773474
28.6719624	−21.4	0.00056923	−3.2447138
28.8369766	−21.5	0.00066640	−3.1762653

The corresponding error surface for Solver again exhibits a pronounced collinearity, as also indicated by the value of −0.96 for $r_{01} = r_{10}$ found in cells F9 and E10 in Figure 12.

■ THE ERROR SURFACE, ONCE MORE

We are used to the idea that the minimum value of SSR in least-squares analysis is a narrow funnel, with a bottom resembling an elliptic paraboloid, quite unlike the trench-like appearance of the error surface in Figure 4. Can we explain its apparently linear shape? Moreover, if its bottom follows a straight line, then what parameters define its apparent slope and intercept?

To answer these questions, we add to the spreadsheet of Figure 2 in cell E4 the instruction =$C$8+$D$8*D4, copy this instruction to cells E5:E7, with a descriptive label such as $(\ln k)_{calc}$ in cell E1. In cell G7, we compute SSR as =SUMXMY2(C4:C7,E4:E7). We can then change the value of a_1 in cell D8, and use Solver to minimize SSR with only one adjustable variable, a_0 in cell C8, to furnish us the minimum value of SSR for a particular E value. Table 2 shows some results in the close neighborhood of the minimum.

We see that the trench has a well-defined though quite shallow minimum, which we could not quite discern in Figures 6 because its vertical scale is logarithmic. In Figure 13, we reproduce the error surface of Figure 4 with superimposed, additional data points to those of Table 2, and the straight line drawn through them. Fitting this straight line through all 34 points used in Table 2 and Figure 13 yields $a_0 = -6.6410675232_3 \pm 0.0000000004_4$ and $a_1 = -1.65014158517_1 \pm 0.00000000002_0$, with $s_f = 3._8 \times 10^{-11}$, indicating an excellent linear fit.

What is the physical meaning of this intercept and slope? For a_0, compare its value with that of ln k for the centered approach in cell C25 of Figure 3, and for a_1, consider the value of $(1/RT)_{av}$ in cell G19 of that same figure. Thus, the error surface of the noncentered approach contains in the coordinates of its "trench" the essential components of its centered counterpart! We indeed have a "pit" in the shape of an ellipsoidal paraboloid, but its horizontal cross section is a *very elongated* ellipse,[32] oriented along the independent parameter $-(1/RT)_{av}$. Sillén's "pit" is now a narrow groove.

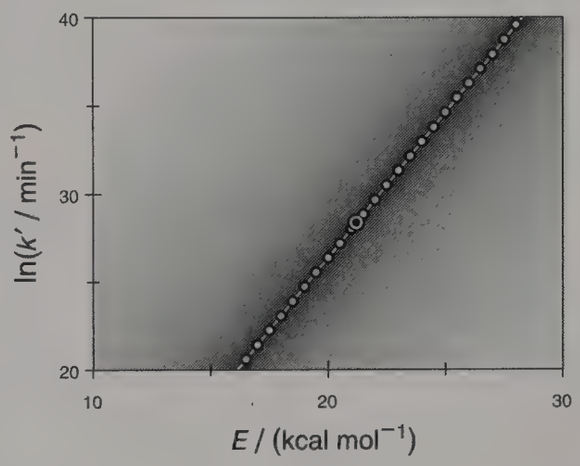

Figure 13. The error surface of Figure 4 with superimposed discrete points (white) displaying data such as listed in Table 2 but obtained over a wider range. The minimum value of log SSR in Figure 2 is emphasized as a larger point with a black center.

Figures 4 and 6 vividly illustrated the nature of collinearity, viz., that it can seriously undercut the specificity of the least-squares criterion SSR, because the effect on SSR due to a variation in one coefficient can be largely compensated by a corresponding change in another coefficient. In Figure 6 we see that, outside the trench, log SSR exceeds −1, while in its center it is nearly constant and well below −3, see Table 2, that is, SSR is at least 2 orders of magnitude smaller. As a consequence, the computed values of both coefficients can readily be shifted along the axis of the trench by relatively small amounts of experimental noise. This is why collinearity can be such a nasty problem. For example, it makes the NIST StRD (*st*andard *r*eference *d*ata) test set Filip difficult or impossible to solve with many standard least-squares algorithms,[25] and can also lead to completely erroneous conclusions,[33,34] as pointed out in, for example refs 9, 26, section 2.18, 32, 35, and 36.

■ DISCUSSION

You may well wonder why we have used such a small sample, of just four data points, for this elaborate demonstration. One

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reason is that such a limited data set tends to put the emphasis on the data analysis *method*, rather than on the data themselves, and also allows us to display the entire spreadsheet. Moreover, in part because its data range is so restricted, we obtain a rather dramatic 50-fold increase in standard deviation when we compute it incorrectly. At any rate, while a four-point data set is limited, it is not unusually small for this type of undergraduate lab experiment, although one would hope that it would normally cover a wider temperature range. At its time, it was certainly good enough to be published in the *Journal of the American Chemical Society* (*JACS*).

We have used Excel to make and illustrate our point, but the following conclusions are not restricted to this or any other specific software system. Moreover, we have used general-purpose, readily available software in order to focus on the approach rather than on its specific computer implementation. For those interested in the latter, the open-access nature of some of the routines used here[24] make them fully transparent and even user-modifiable.

Collinearity in the *output parameters* of least-squares analysis can corrupt estimates of propagated uncertainty calculated from their standard deviations. When subsequent results are derived from two or more such parameters, much more serious errors can result, as illustrated in ref 26, section 2.18. In the presence of severe output collinearity, as with the NIST test data set Filip, the model parameters themselves may be so strongly compromised that they no longer contain any significant digits.[18]

We can qualitatively understand the collinearity problem illustrated here in terms of extrapolation. The data of Bruice and Schmir[24] cover a quite restricted temperature range, of only 14 °C, which is small indeed on the inverse absolute temperature scale, where $1/RT$ varies only between 1.61 and 1.69. The least-squares model then yields the slope a_1 (which is independent of the absolute values of $1/RT$), and the intercept a_0, that is, the value of y at $1/RT = 0$ (where T approaches infinity). This involves a long extrapolation, approximately 200 times the x range of the experimental data. The resulting value of a_0 is therefore highly leveraged; that is, a small amount of noise in one of the points of the experimental data set can have an inordinately large effect on the value of a_0, and this is enhanced in our case because there are so few data points to counterbalance a single random error, especially one at either end of the data range. Using centering avoids the long extrapolation, because the intercept is now at $x' = x - \Sigma x$, which always lies *within* the data set. Centering therefore replaces an extrapolation by a much less error-prone interpolation. Alternatively, the covariance can be used (if provided by the software) to find the proper way back from such a long extrapolation. Incidentally, the above example illustrates the danger of relying solely on R^2, in this case 0.9997 (see cell F12 in Figure 2 and F29 in Figure 3), a feel-good measure that looks at the correlation between the *input* data, but cannot alert us to any potential problems with the *output* of the least-squares fit, a much more common occurrence in chemical data analysis.

So far we have only considered the straight line $y = a_0 + a_1x$, and we have seen a simple way to avoid collinearity of a_0 and a_1 through centering the independent variable x. By substituting $x' = x - \Sigma x$, this leads to $y = a_0' + a_1x'$, where $a_0' = a_0 + a_1\Sigma x$. In other words, we fit the experimental data to $y = a_0' + a_1x'$, and from the resulting parameters a_0' and a_1 compute both $a_0 = a_0' - a_1\Sigma x$ and $x = x' + \Sigma x$; in the case of weighted least-squares, we used centered weighting.

In Weighted Least Squares, we stated that the original data used in our example were k-values. Literally this is correct, but the original kinetic observations were of concentrations c as a function of time t. From these, the k-values were most likely obtained from plots of ln c versus t, thereby introducing another data transformation step. However, since no information is available on the experimental uncertainties in c or k, we have merely taken the k-values as our primary input data, at face value. Students performing the entire experiment, from isothermal kinetic concentration—time profiles to computing the standard rate constant and activation energy, should of course first use centered weighted linear least-squares or nonlinear least-squares (and preferably both) to analyze their kinetic data, then use the so obtained uncertainty estimates in their k-values for the subsequent thermodynamic analysis.

The proportionality $y = a_1x$ has no collinearity problems, because it contains only one model parameter, a_1. Centering works for a straight line, but not for polynomial or multivariate least-squares, for which one needs orthogonal polynomials. However, the coefficients resulting from such a fit in terms of orthogonal functions need to be converted back to apply to the system parameters, and this can make the operation much more laborious and time-consuming than using the covariance matrix.

Interestingly, a value close to 1 for the linear correlation coefficient $|R|$ or R^2 between two input parameters is usually considered desirable, while it is not a good sign when $|r_{01}|$ approaches 1, because it indicates strong collinearity between those output parameters. While a stronger linear correlation between the *input* parameters may satisfy our preference for the simplicity of a linear relationship, a stronger linear correlation between the *output* parameters makes the matrix $\mathbf{X}$ approach singularity, and therefore corrupts the precision, and ultimately even the accuracy, of the traditional methods to solve the least-squares problem.

The data set analyzed here, with its strong mutual dependency between a_0 and a_1, with $r_{01} = -0.99985$, clearly illustrates the efficiency of either centering or using the covariance matrix. Eventually, with sufficiently strong collinearity, the adjustable parameters will also be affected seriously, in which case we will need to use a least-squares routine that is rather immune to collinearity, such as one based on either QR matrix decomposition (used in LinEst and Regression since Excel 2003) or on singular value decomposition, or a routine that uses quadruple or higher numerical precision to find accurate least-squares coefficients a_i. Even then we will still need to use the covariance matrix to compute the precision of any results that are derived from two or more of the resulting least-squares parameters.

It may be useful to stress again that the covariance cannot be ignored as a mere higher-order effect such as, for example, skew or kurtosis. The covariance is of the very same order as the variance, and its effect on the standard deviation is fully equivalent to that of a variance. To use one and ignore the other merely distorts the answer.

Manually computing the covariance matrix, and using it subsequently to calculate a resulting propagation of imprecision, is usually quite cumbersome, because it involves partial derivatives, but letting the computer do this tedious work is simple and fast, and the software is readily available.[24] Moreover, its open access makes it transparent for those who want to look under the hood, and the price is right: it is free for educational and research purposes. Of course, using such software still requires an understanding of what is involved, just as we do not need to know how

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to compute a logarithm in order to use it, as long as we understand what it means and does. In view of the greatly increased use of least-squares analyses in the past few decades, chemistry textbooks should be upgraded to include a discussion of covariance and collinearity, though not necessarily the mathematical details of its computation.

It is unfortunate that the NIST reference data sets do not provide the covariance matrix, because this might spur software developers to do so as well. The makers of the NIST test data sets were well aware of collinearity, and clearly designed their data set Filip, the test most often flunked by commercial linear least-squares software, to highlight this specific problem.[18] Shortly after the introduction of least-squares by Legendre (1805)[37] and Gauss (1809),[38] the latter included the covariance matrix as part of his theory of the "reciprocal matrix" (Gauss 1828).[20] Laplace, who contributed the most general foundation of least-squares through his central limit theorem,[39] apparently already knew in 1827 how to deal correctly with multiple least-squares parameters with correlated errors and a known covariance structure.[40]

At a more popular level, the need to consider collinearity and the requisite mathematical operations were described by, for example, de Forest Palmer in his 1912 *The Theory of Measurements*,[41] by Fisher in his 1925 *Statistical Methods for Research Workers*,[42] in Deming's influential *Statistical Adjustment of Data*[43] where it was called the "product variance", and in countless other statistical textbooks and manuals, including NIST's own *e-Handbook of Statistical Methods*.[44] The latter, in its section 2.5.5 on "Propagation of error considerations", states that "Generally, reported values of test items from calibration designs have *non-zero* covariances that must be taken into account ...". In its section 2.3.6.7.1, it illustrates the quite significant errors one can make by ignoring the covariances, and states that, "Unfortunately, some statistical software packages do not display these covariance terms with the other output from the analysis."

It is therefore incomprehensible why NIST, as the practical standard-setter in this case (the *s* in both NBS and NIST), has so far not included covariances in its own statistical reference data sets. Yet, as long as NIST does not lead in this area, few commercial software developers will follow.

In the example used here, we had to consider the propagation of uncertainty to compute the standard deviation of k_{25} from all but one of the linear least-squares analyses used, but that was not required when we used nonlinear least-squares. Why was this so?

The answer lies in how the problem is formulated. When we use expressions such as eq 10 or 11, we need to use both slope and intercept of the resulting least-squares results to compute k_{25}, and therefore must use the proper method of propagation of imprecision. However, by formulating the problem so that k_{25} or $\ln k_{25}$ is determined directly, as in eq 12, we avoid the latter step, and obtain its standard deviation directly. This is often easier to do with nonlinear least-squares but, regardless of the method used, requires that some thought be given, in advance of the analysis, as to which parameters we really want to determine, and how we can do so most efficiently.

The macros LS, WLS, and SolverAid all generate the covariance matrix, and Propagation can read it, making routine application of the methods illustrated here virtually effortless. Since almost all least-squares analyses are done by computer anyway, there is every reason to use the covariance matrix in general for all problems involving the propagation of uncertainty, to explain its underlying principles, and to use it in practice in the undergraduate chemistry curriculum. Incidentally, it may also avoid much frustration on the part of students in physical chemistry lab, who often balk at laborious "error analysis" requirements in their laboratory reports that may well distract them from the main purpose of the experiment. Little do they know that their elaborate "error analysis" is all too often a fool's errand, because they may not even have been given the (statistical and software) tools they would need to do it properly.

■ SUMMARY

This article emphasizes the need to consider the covariances together with the usual variances in the propagation of parameter uncertainty of derived quantities and uses a simple data set from the literature to illustrate its applicability. We demonstrate three specific ways to handle the covariance. For linear as well as nonlinear least-squares, the most general approach is to use software that computes and displays the variance—covariance matrix, in conjunction with error propagation software that can then utilize this information. Another general approach is to reformulate the problem, so that it computes the desired parameter(s) directly, thereby avoiding the need for error propagation. This can often be accomplished with linear as well as nonlinear least-squares. Finally, for straight-line relationships, centering can be used to make the covariances zero. When weighted least-squares are used to fit data to a straight line, *weighted* centering is necessary for the covariance to be zero. All the above approaches are illustrated on a spreadsheet, both numerically and graphically.

■ ASSOCIATED CONTENT

Supporting Information

Appendices A and B. This material is available via the Internet at http://pubs.acs.org, and in ref. 24.

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ARTICLE

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Nonisothermal Analysis of Solution Kinetics by Spreadsheet Simulation

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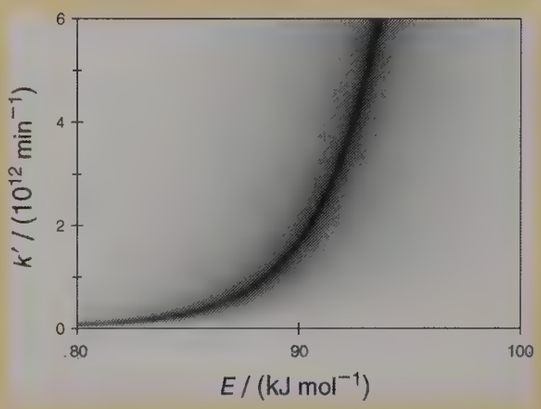
Supporting Information

ABSTRACT: A fast and generally applicable alternative solution to the problem of determining the useful shelf life of medicinal solutions is described. It illustrates the power and convenience of the combination of numerical simulation and nonlinear least squares with a practical pharmaceutical application of chemical kinetics and thermodynamics, validated by comparison with literature data.

KEYWORDS: Graduate Education/Research, Upper-Division Undergraduate, Physical Chemistry, Computer-Based Learning, Aqueous Solution Chemistry, Drugs/Pharmaceuticals, Kinetics, Reactions



Textbooks are traditionally and justifiably conservative, and kinetic analysis is typically illustrated by isothermal kinetic measurements that validate kinetic laws and provide the corresponding reaction rate constants. By combining these rate constants for several such measurements on the same reacting system at different temperatures, one can then compute the corresponding activation energy, provided that the mechanism stays the same. The preceding article[1] illustrates the proper analysis of isothermal reaction kinetics, which is not always quite as straightforward as it is sometimes made out to be.

Industrially, however, the above, two-stage approach is often considered too time-consuming, and more efficient shortcuts are sought. One of these is to use nonisothermal kinetic analysis of, for example, medicinal solutions, for which the decomposition kinetics need be known in order to establish their useful shelf life.

This is now a well-established approach, introduced to pharmacology by Rogers,[2] who used an inverse-logarithmic temperature−time profile that made the analysis of first-order irreversible kinetics mathematically tractable. It was soon followed by the use of a reciprocal heating regime.[3] Further development included solving the equation for a linear temperature increase with time, and then fitting the data to resulting sets of model curves based on assumedly precise first and last data points.[4,5] Alternatively, the problem was approached by numerical integration[6] or by differentiation.[7,8] A brief overview of the early development of these different approaches was given by Kipp.[9]

The *combination of digital simulation with nonlinear least-squares optimization* yields yet another approach, with the advantages of conceptual simplicity, direct comparison of the model and the raw experimental data, complete flexibility in terms of temperature profile and model assumptions, and ease of implementation. Moreover, it is a good illustration of the general power of the combination of numerical simulation with nonlinear least squares, a quite general approach that can solve many mathematically well-posed problems that lack suitable closed-form analytical solutions. As our software we will use the Excel spreadsheet, underscoring the inherent simplicity and general availability of this approach.

We will consider a unidirectional (i.e., irreversible) first-order decomposition of the active ingredient of initial concentration c_0 in a medicinal solution, so that

$$\frac{dc}{dt} = -kc \qquad (1)$$

where c is concentration, t is time, and k is the rate constant of the reaction, with $c = c_0$ at $t = 0$. This model is not chosen for its mathematical simplicity (which is irrelevant in a digital simulation), but because all literature examples we have found happen to involve this simplest of all kinetic mechanisms. There is therefore no need to go beyond first-order kinetics in this article, but also no difficulty to extend the model to more complicated kinetics if this were required.

We assume that the absolute temperature T is kept constant for a time period Δt, and is then raised in stepwise fashion to $T + \Delta T$. For the concentration c, we will use the semi-implicit Euler method,[10] which approximates the concentration c on the right-hand side of eq 1 during that period Δt as $c + \Delta c/2$, that is, as the *average* of the initial and final reagent concentrations, c and $c + \Delta c$, respectively, in that short interval. The implied assumption here is that, over a sufficiently short interval, the concentration change is a nearly linear function of time t. The slightly simpler explicit Euler approximation,[10] which takes the average concentration c over that small interval Δt as equal to its *initial* value, is

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not used here because it sometimes caused oscillatory behavior. Not only is it less stable, it is also less accurate than the semi-implicit method. In both cases, eq 1 is approximated by the corresponding difference equation $\Delta c/\Delta t = -kc$.

We therefore use

$$\frac{\Delta c}{\Delta t} = -k\left(c + \frac{\Delta c}{2}\right) \tag{2}$$

so that identifying c with c_{old} and $c + \Delta c$ with c_{new} yields

$$c + \Delta c = \frac{1 - k\Delta t/2}{1 + k\Delta t/2}c \quad \text{or}$$

$$c_{new} = \frac{1 - k\Delta t/2}{1 + k\Delta t/2}c_{old} \tag{3}$$

where, in this case of decomposition, Δc will be a negative quantity.

For the dependence of the rate constant k on the absolute temperature T, we will assume the Arrhenius equation

$$k = k' \exp\left(\frac{-E}{RT}\right) \tag{4}$$

where k' is a constant (sometimes called the "frequency factor"), E is the activation energy of the reaction, and the gas constant R has the value 1.9858775×10^{-3} kcal mol^{-1} K^{-1}.

Now assume that the temperature, after having been kept constant for a period Δt, is raised abruptly to $T + \Delta T$. To compute the rate constant $k_{T+\Delta T}$ at that new temperature, we write eq 4 twice, first for T and then for $T + \Delta T$, and find its ratio as

$$\frac{k_{T+\Delta T}}{k_T} = \frac{k' \exp\left[\dfrac{-E}{R(T + \Delta T)}\right]}{k' \exp\left(\dfrac{-E}{RT}\right)}$$

$$= \exp\left[\frac{-E}{R}\left(\frac{1}{T + \Delta T} - \frac{1}{T}\right)\right] \tag{5}$$

so that

$$k_{T+\Delta T} = k_T \exp\left[\frac{E}{R}\left(\frac{1}{T} - \frac{1}{T + \Delta T}\right)\right] \quad \text{or}$$

$$k_i = k_0 \exp\left[\frac{E}{R}\left(\frac{1}{T_0} - \frac{1}{T_i}\right)\right] \tag{6}$$

where the reference temperature k_0 is often taken at a convenient temperature. By making ΔT and Δt sufficiently small, this stepwise, discontinuous approach can also be used to approximate a gradual, continuous temperature change.

Below we will therefore use a sequence of discrete periods of constant duration Δt, during each of which the temperature is kept constant at a value ΔT above that applied during the previous interval Δt. We start with the initial concentration c_0 and rate constant k_0 at the initial temperature T_0, which is maintained for a time Δt, during which the amount decomposed is given by $\Delta c = k_0 c\Delta t/[1 + k_0\Delta t/2]$. For the next interval we repeat this, but with $c_0 + \Delta c$ as our new starting concentration, and with the value of $k_{T+\Delta T}$ appropriate for the new temperature $T + \Delta T$. This iterative process is repeated until we have reached the final temperature or a constant (in this case: zero) concentration c. The required relations only need to connect the molecular response in one time interval to that during the immediately preceding period, a convenient situation for a numerical simulation.

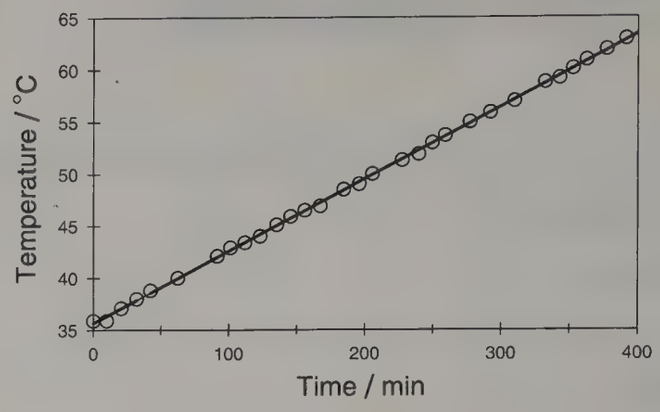

Figure 1. The relationship between temperature and time reported by Kipp (open circles) and a straight line (drawn) fitting these data using least-squares analysis.

A FIRST EXAMPLE

In a study of the acid-catalyzed hydrolysis of *p*-nitrophenyl acetate, Kipp[9] took occasional samples of the reaction mixture at different times and temperatures for subsequent analysis by liquid chromatography, which yielded separate peaks for *p*-nitrophenyl acetate and for its hydrolysis product, *p*-nitrophenol. From these, he determined the concentration fractions c/C of the unreacted *p*-nitrophenyl acetate, where c is its concentration and C the sum of the concentrations of *p*-nitrophenyl acetate and *p*-nitrophenol. We will here use the set of experimental data reported by Kipp in his Table 3.

For our simulation, it is convenient to place, at the top of an Excel spreadsheet, say in cells C3:C5, the three assumed parameters, that is, in cell C3 the initial concentration fraction c_0/C of *p*-nitrophenyl acetate (where c_0 is the value of the concentration c at the beginning of the experiment), in cell C4 the reaction activation energy E (in units of kcal mol^{-1}), and in C5 the reference rate constant k_{25} at 25 °C (in min^{-1}). Because a convenient layout of the spreadsheet is a significant organizing (and time-saving) part of the procedure, we will describe it here in some detail.

Leaving rows 6−15 open for subsequent results and column headings, we copy the data of Kipp's Table 3 to the spreadsheet, using A16:A46 for time t, with the corresponding temperatures, in °C, ranging from 35.9 to 64.1 °C, in B16:B46. In C16:C46, we then compute $1/RT$, where $R = 0.0019858775$ kcal mol^{-1} K^{-1}, and T is 273.15 plus the temperature in °C. Moreover, it is convenient to compute $1/RT_{25}$ =1/(0.0019858775*298.15) in cell G3, with a label in cell F3. In D16, we place the instruction for the rate constant k as =$C$5*EXP($C$4*($G$3-C16)), and again copy this down all the way to row 46. Finally, we enter Kipp's experimental concentration ratios c/C in E16:E46.

The dependence of temperature (in B16:B46) on time (in A16:A46) is illustrated in Figure 1. Linear least-squares analysis shows that it is described quite well by a linear dependence of the listed temperature (in °C) on reaction time t, that is, as $a_0 + a_1 t$. The parameters found are $a_0 = 35.66_7 \pm 0.06_3$ and $a_1 = (0.0692_6 \pm 0.0002_7)$ °C^{-1}, with a standard deviation of the fit, s_b of

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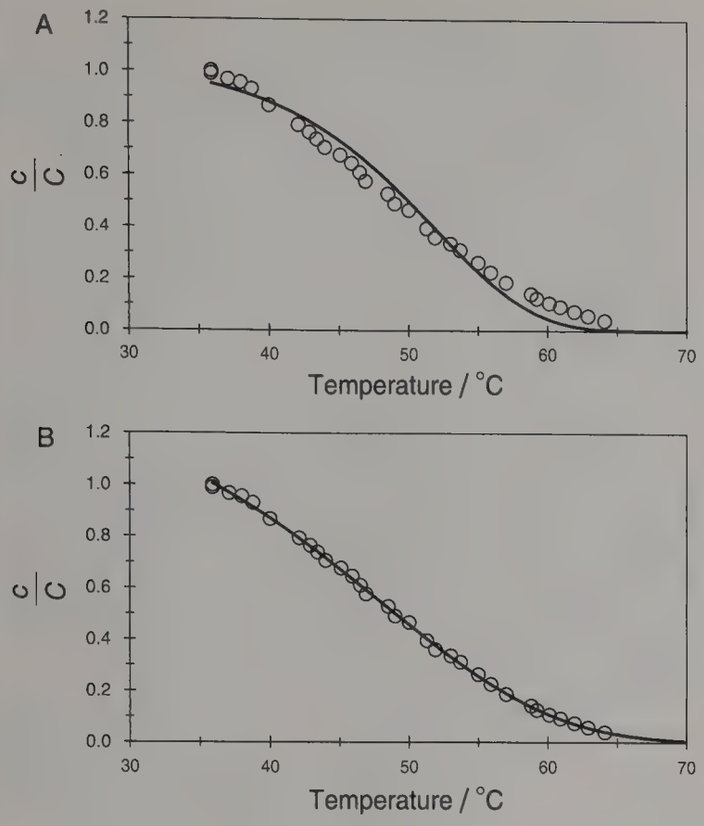

Figure 2. The experimental data reported by Kipp (open circles) and the corresponding, simulated data after (A) rough visual adjustment of the assumed fitting parameters to $c_0/C = 0.95$, $E = 30$, and $k_{25} = 1.5 \times 10^{-4}$ and (B) after minimizing the sum of squares of the residuals with the nonlinear least-squares macro Solver.

0.18_2 °C. A quadratic term a_2 (the usual way to represent slight nonlinearity) is unwarranted, as it would have a standard deviation $s_2 = 2.4 \times 10^{-6}$, which is larger than the absolute value $|a_2| = 2.1 \times 10^{-6}$ of the coefficient a_2 itself. For the inverse relation of time t as a function of temperature, we find t in °C as $(-514._7 \pm 2._8) + (14.43_2 \pm 0.05_6) \times (T - 273.15)$. Returning to the top of the spreadsheet, in cell F4 place the label $\Delta t/2$=, and in cell G4 deposit the corresponding instruction =(A60-A59)/2. We are now ready to start the simulation.

Separated by 12 empty rows, we extend the second column, again starting with 35.9 °C in cell B59 and ending well beyond 64.1 °C, say at 70.0 °C in cell B400, but now with constant increments of 0.1 °C. In A59:A400, we then compute values for $t = -514._7 + 14.43_2$ times the corresponding temperatures in °C, in C59:C400 the associated values of $1/RT$ as in C16:C46, and in D59:D400 the corresponding k values as in D16:D46. To keep round-off errors to a minimum, we use the spreadsheet-generated results for the best-fitting least-squares line directly (which happens to be in cells $R$73 and $S$73 on my spreadsheet) rather than those rounded values of $-514._7 + 14.43_2$. In F59, we place =C3, in F60 the instruction =F59*(1-D59*$G$4)/(1+D59*$G$4) for the concentration fractions c/C based on eq 3 and the assumed initial concentration fraction c_0/C. Copy this down to F61:F400.

We now refer in G16:G46 to the simulated c/C values in F59:F400 at the corresponding temperatures in B59:B400. We have used the 12-row offset to facilitate connecting temperatures in B16:B46 with the corresponding values in B59:B400. In G16 and G17 (for a temperature of 35.9 °C in B16 and B17), place the instruction =F59, in G18 for 37.1 °C use =F71, in G19 for

A

	A	B	C	D	E	F	G	H
1	Analysis of the data of J. E. Kipp, *Int. J. Pharmacoceutics* 26 (1985) 339-354.							
2								
3			c_0/C =	1.00548621	4.2242E-03		$1/RT_{25}$ = 1.688934	mol kcal^{-1}
4			E =	16.5949387	3.8513E-01	kcal mol^{-1}	$\Delta t/2$ = 0.721593	min
5			k_{25} =	7.5002E-04	3.4392E-05	min^{-1}	SSR = 0.00207018	0.0085986
6		CM:	1.78441E-05	-9.44E-04	9.5789E-08			
7			-0.000943636	0.1483268	-1.306E-05			
8			9.57889E-08	-1.306E-05	1.1828E-09			
9		CC:	1	-0.5800257	0.65934			
10			-0.580025664	1	-0.98593			
11			0.65933563	-0.985935	1			
12								
13	t	T-273.15	$1/RT$	k			c/C_{exp}	c/C_{calc}
14	min	°C	mol kcal^{-1}	min^{-1}				
15								
16	0.00	35.9	1.629367	2.0155E-03			1.000000	1.005486
17	10.00	35.9	1.629367	2.0155E-03			0.990050	1.005486
18	20.63	37.1	1.623064	2.2377E-03			0.967200	0.969314
19	31.97	38.0	1.618370	2.4190E-03			0.956060	0.940575

B

	A	B	C	D	E	F	G	H
1	Analysis of the data of J. E. Kipp, *Int. J.. Pharmacoceutics* 26 (1985) 339-354.							
2								
3			c_0/C =	1 0054862	0.000458996		$1/RT_{25}$ = 1.688934	mol kcal^{-1}
4			E_c =	16.6948932	0.038327199	kcal mol^{-1}	$\Delta t/2$ = 0.721593	min
5			$k_{c\,25}$ =	7.1116E-04	3.36265E-06	min^{-1}	SSR = 0.0020716	0.0024719
6		CM:	2.107E-07	-1.154E-05	1.12267E-09			
7			-1.154E-05	0.001469	-1.27225E-07		E_w = 14.29	kcal mol^{-1}
8			1.123E-09	-1.272E-07	1.13074E-11		$k_{w\,25}$ = 3.57E-05	min^{-1}
9		CC:	1	-0.6558911	0.727384126		N = 1	
10			-0.6558911	1	-0.987152389			
11			0.7273841	-0.9871524	1			
12								
13	t	T-273.15	$1/RT$	k			c/C_{exp}	c/C_{calc}
14	min	°C	mol kcal^{-1}	min^{-1}				
15								
16	0.00	35.9	1.629367	2.006155E-03			1.00000	1.005486
17	10.00	35.9	1.629367	2.006155E-03			0.99005	1.005486
18	20.63	37.1	1.623064	2.227335E-03			0.96720	0.969291
19	31.97	38.0	1.618370	2.407821E-03			0.95606	0.940536

Figure 3. The top of the spreadsheet (A) annotated with some labels and dimensions, after use of Solver and SolverAid and (B) after the refinements described in the text.

38.0 °C deposit =F80, and so on. Then, plot the experimental data in F16:F400 and the calculated ones in E16:E400 as a function of temperature in B16:B400.

We can use this graph to manually adjust the assumed values for c/C, E, and k_{25}, in order to make the simulated curve visually coincide *roughly* with the experimental data, as illustrated in Figure 2A. We can easily obtain a better manual fit than is shown here, by fiddling some more with the parameters, but a crude manual fit such as shown here is usually all that is required.

After all this preliminary work, we are now ready for the actual data fitting. In cell G5, we use the Excel function SumXMY2 (F16:F46,G16:G46), an instruction that is easy to remember as ***sum*** of (***x*** ***m***inus ***y***) to the power **2**, to compute SSR, the ***s***um of the ***s***quares of the ***r***esiduals, where the residuals are the differences between the experimental and corresponding simulated y values. We use Solver to minimize that sum of squares of residuals in G5, the Solver "target", by adjusting the parameter values for c_0/C, E, and k_{25} in C3:C5. Because the adjustable parameters differ by orders of magnitude, we must use Solver's automatic scaling option. Starting from different, visually plausible values results in essentially identical answers. The result is shown in Figure 2B.

We now use SolverAid[11,12] to provide the standard deviation of the overall fit of the model to the data (in cell H5); the standard deviations of c_0/C, E, and k_{25} (in D3:D5); and the associated covariance matrix and correlation coefficients (in B6:D8 and B9:D11, respectively). The top of the spreadsheet will now look like that shown in Figure 3A.

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Table 1. The Results of Changing the Number of Equidistant Time and Temperature Steps, N_s, within the Spreadsheet Intervals Used in Rows 59−400, which for N_s = 1 Were Δt = 1.4432 min and ΔT = 0.1 °C

N_s	c_0/C	E_c /(kcal mol^{-1})	$k_{c,25}$/(10^{-4} min^{-1})[a]
1	$1.0054_9 \pm 0.0004_3$	$16.68_1 \pm 0.03_5$	$7.15_4 \pm 0.03_2$
10	$1.0054_9 \pm 0.0004_6$	$16.69_5 \pm 0.03_8$	$7.10_9 \pm 0.03_4$
100	$1.0054_9 \pm 0.0004_6$	$16.69_5 \pm 0.03_8$	$7.11_2 \pm 0.03_4$

[a] In the present case, using 100 times smaller steps only has a minor effect on $k_{c,25}$.

■ REFINING THE MODEL BY INCORPORATING A COMPETING REACTION

The above model might be incorrect or applied incorrectly. In this case, a more complete model should include the uncatalyzed reaction and make sure that the intervals Δt and ΔT used are small enough to avoid significant systematic errors. Only after we have tied down these two possible "loose ends" can we be reasonably confident of the results. Below we will make the corresponding adjustments. Note that we need not use global weighting[13] because the parameters c_0/C, E, and k_{25} are determined directly using nonlinear least-squares, so that no transformation of variables is involved.

To correct for the uncatalyzed hydrolysis of *p*-nitrophenyl acetate, Kipp merely subtracted the rate constant k_{25} of the uncatalyzed reaction, as determined by Tucker and Owen,[14] from his computed result to obtain corrected rate constants. This cannot serve as a general method, although it is most likely adequate in this particular case, because Tucker and Owen reported the rate constant of the uncatalyzed reaction at 25 °C as 1.096×10^{-5} s^{-1}, and its activation energy as 14.29 kcal mol^{-1}, which are both smaller than the corresponding values for the catalyzed process. The lower activation energy of for the uncatalyzed process is of course improbable, and most likely reflects the different experimental conditions used by the different authors. Nonetheless, we here will use the same numerical values that Kipp used (even if their numerical compatibility is questionable) to indicate how, in general, an additional parallel reaction can be incorporated properly in the simulation.

We therefore consider two reactions, with in this case are the (pseudo-)first-order catalyzed reaction with a rate constant k_c, and the first-order uncatalyzed process with rate constant k_u, together with their activation energies E_c and E_u, respectively. We replace k in eqs 1−3 by $k_c + k_u$, as appropriate for two parallel reaction pathways, and use

$$k_i = k_{c,25} \exp\left[\frac{E_c}{R}\left(\frac{1}{T_{25}} - \frac{1}{T_i}\right)\right] + k_{u,25} \exp\left[\frac{E_u}{R}\left(\frac{1}{T_{25}} - \frac{1}{T_i}\right)\right] \quad (7)$$

to compute their values at different temperatures T_i.

We use the same values as used by Kipp, viz., E_u = 14.29 kcal mol^{-1} and $k_{u,25} = 3.791_4 \times 10^{-5}$ min^{-1} derived from measurements by Tucker and Owen at 65 °C.[14] To implement this, we enter these values in cells G7 and G8, respectively, with corresponding labels in F7:F8, then modify the instruction in cell D16 to read =$C$5*EXP($C$4*($G$3-C16))+$G$8*EXP($G$7*($G$3-C16)), and copy this to D17:D46 and to D59:D400. Note that this has only a minor effect on the resulting k values in Figure 3B, because $k_{u,25}$ is about 20 times smaller than our estimate for $k_{c,25}$ and, more importantly, E_u is also smaller than E_c so that, relative to the catalyzed process, the uncatalyzed reaction becomes even less important at higher temperatures.

With the above changes, run Solver again, followed by SolverAid. We now find for the effective, overall rate constant k_{25} the value $(7.15_4 \pm 0.03_2) \times 10^{-4}$ min^{-1}, and for the effective activation energy $E = 16.68_1 \pm 0.03_5$ kcal mol^{-1}, only slightly different from our earlier result.

■ USING SMALLER STEPS IN TEMPERATURE AND TIME

The second refinement concerns the step size Δt. We already replaced the unevenly spaced experimental data (in rows 16−46) by data at regular time and temperature intervals (in rows 59−400), and in principle could do the same at smaller intervals, by extending the column lengths. It is far easier, however, to leave the spreadsheet intact, and instead to use a simple custom function, as shown in the Appendix (see the Supporting Information), to perform the computation in smaller time increments.

We substitute this function, which we will call NStep1, for the instruction in E60, then copy it down to row 400. The actual instruction entered in E60 should read =NStep1($G$9,A59,A60, $R$73,$S$73, D59, E59,$C$4,$C$5,$G$7,$G$8). Here cell G9 contains the value 1 for the number of steps per interval, N_s, and $R$73 and $S$73 refer to the cells containing the least-squares slope and intercept of the dependence of time (in minutes) on temperature (in °C) on my spreadsheet (these latter two addresses are most likely different on yours). The activation energy E_c and reference rate constant $k_{c,25}$ of the catalyzed reaction are in cells C5 and C6, and the corresponding parameters E_u and $k_{u,25}$ for the uncatalyzed process in cells G7 and G8.

After copying this instruction down to row 400, try it. Nothing should happen, because with the first value in the argument of Nstep1, N_s = 1, you specified just one step, in which case the result should be identical to that obtained without the function. Then, try N_s = 10, use Solver and SolverAid, and see whether the result has changed significantly. If so, try N_s = 100, and so forth, until you get a stable reading of all the significant figures in your result. (With so many extra steps, the computation will slow down noticeably.) You can of course use different integer numbers of steps, such as N_s = 2, 4, 8, 16, ... Table 1 summarizes some results, and Figure 4 shows the final spreadsheet for N_s = 100. In principle, we could also use the spreadsheet to determine E_u and $k_{u,25}$ from the experimental data, but in practice that would be nearly impossible, because $E_u \ll E_c$ and $k_{u,25} \ll k_{c,25}$, especially in view of the pronounced collinearity of the system, see Figure 4.

■ COMPARISON WITH LITERATURE DATA

To compare our result with those in the literature, divide the rate constant $k_{c,25} = (7.11_2 \pm 0.03_4) \times 10^{-4}$ min^{-1} by 60 s min^{-1}, and by the reported hydrogen ion concentration [H$^+$] of $(800/810) \times 0.25 = 0.246_9$ mol L^{-1}, to yield $k/[H^+] = (4.80_1 \pm 0.02_3) \times 10^{-5}$ L mol^{-1} s^{-1}. As can be seen in Table 2, the results obtained here agree well with the literature values. The correlation coefficients in cells C11 and D10 show significant collinearity between E and k_{25}, as is typical of this type of problems.[1]

For ease of comparison, we have listed in Table 2 the activation energy and rate constant of the catalytic reaction. While these and their counterparts for the uncatalyzed reaction

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are the chemically most meaningful quantities, their combined effect is probably more relevant for defining the solution stability, perhaps in the form of "effective" kinetic parameters E_{eff} and k_{eff}.

A final comment: the pseudo-first-order rate constant $k_{25}/[H^+]$ of the acid-catalyzed reaction in the first column of Table 2 has a value similar to the first-order rate constant k_{25} reported by Bruice and Schmir,[16] and their activation energy is actually significantly higher. These measurements, obtained near room temperature in a 5.4 mM phosphate buffer at pH 8 containing 28.5% (v/v) ethanol, are clearly incompatible with the values listed in Table 2. The results of Kipp (which were the basis of our analysis) were for aqueous 0.247 M HCl containing only 1.25% (v/v) ethanol. This difference in the solvent used is possibly responsible for the discrepancy.

Our analysis of Kipp's experimental data exhibits considerable collinearity between the rate constant k and the activation energy E, see Figure 4 or cells C11 and D10 in Figure 3. Negative correlation coefficients close to −1 were also reported by Kipp.[9] Figure 4 shows the corresponding error surface,[1] that is, log(SSR) for various values of E and k_0, where SSR, the quantity minimized by least-squares and the "target" of Solver, again denotes the *s*um of *s*quares of the *r*esiduals.

■ A SECOND EXAMPLE

As a second example, we consider the data of Madsen et al.[6] on the alkaline degradation of riboflavin, aka vitamin B2. Madsen et al. used a 10^{-4} M solution of riboflavin in 0.1 M NaOH, and took temperature readings (to ±0.5 °C) and reaction samples at fairly regular time intervals. These samples were quenched in acetic acid, and then assayed spectrophotometrically. Figure 5 shows a plot of their temperature−time profile, which least-squares analysis shows to be expressible quite well by the quadratic relationship $T - 273.15 = (20.9_2 \pm 0.2_7) + (0.445_6 \pm 0.009_8)t - (0.00050_3 \pm 0.00007_3)t^2$ where t is time in minutes. Madsen et al.[6] used a seventh-order polynomial, which has an even smaller value of s_f though a higher F value, as readily established with the macro LSPoly.[12] For our purpose, such a high-order fit is not only unnecessary but also undesirable, because it leads to oscillatory interpolations. Both methods kept residuals to within ±0.6 °C.

Using reaction times t from 0 to 165 min, with increments of 0.5 min, we calculate the temperature using this quadratic relationship, and proceed as before, again initially assuming that the temperature remains essentially constant during those intervals Δt. The spreadsheet layout and data analysis procedure are therefore similar to that used previously, the only difference being that Madsen et al. used a standard reference temperature of 20 °C, which we will therefore adapt here as well. Starting from a crude first manual parameter adjustment of $A_0 = 0.7$ absorbance units, $E = 19$ kcal mol^{-1}, and $k_{20} = 0.001$ min^{-1}, see Figure 6A, Solver finds $A_0 = 0.64038_5 \pm 0.00005_1$, $E = 20.257_4 \pm 0.007_9$ kcal mol^{-1}, and $k_{20} = 0.0006469_8 \pm 0.0000009_8$ min^{-1}, as shown in Figure 6B. The resulting values for E and k_{20} again exhibit a considerable collinearity, with a linear correlation coefficient of approximately −0.99. Figure 7 displays the corresponding error surface, log(SSR) as a function of E and k_{20}, with a collinearity that is even more obvious than in Figure 4. Madsen et al. provided equivalent information in numerical form in their Table 5.

Given the more than 5 times faster temperature change than in the previous section, we slightly modify the function Nstep1 to

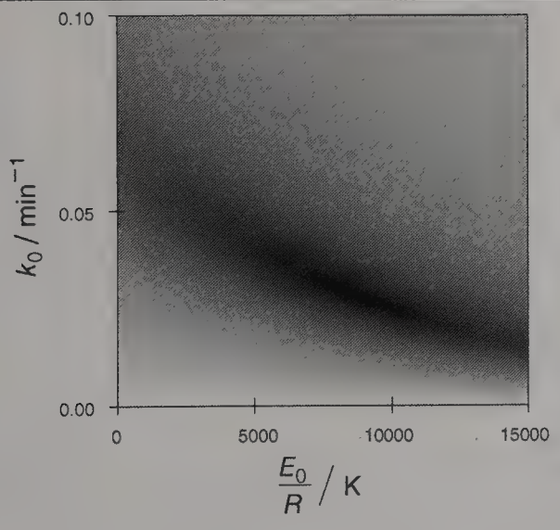

Figure 4. A gray scale error surface (where darker gray represents lower error) for the model used here with the experimental data reported by Kipp.

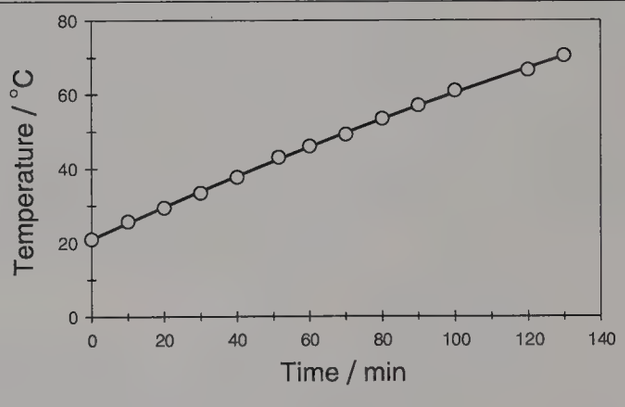

Figure 5. The relationship between temperature and time reported by Madsen et al. (open circles) and a quadratic curve (drawn) fitting these data.

Table 2. Comparison of Reported Results for the Acid-Catalyzed Hydrolysis of *p*-Nitrophenyl Acetate

$k_{25}/[H^+]/(10^{-5}$ L mol^{-1} s$^{-1})$	E/(kcal mol^{-1})	Authors	Ref	Comments[a]
5.3	—	Connors	15	—
—	18	Eriksen and Stelmach	3	*i*
5 ± 1	21 ± 2	Eriksen and Stelmach	3	*ni*
4.75	17.1	Tucker and Owen	14	*i* and *ni* average.
4.1 ± 2.1	17.8 ± 5.7	Kipp	9	*ni*, derivative method
4.4 ± 0.1	17.4 ± 0.5	Kipp	9	*ni*, integration method
4.80 ± 0.02	16.69 ± 0.04	this analysis		*ni*, numerical simulation

[a] *i* denotes isothermal and *ni* nonisothermal.

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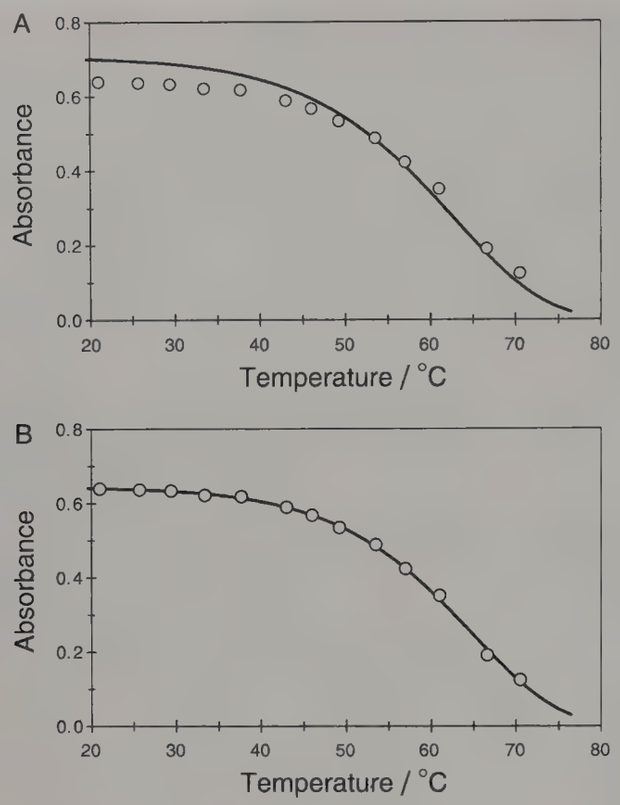

Figure 6. The experimental data reported by Madsen et al. (open circles) and the corresponding, simulated data after (A) rough visual adjustment of the parameters to $A_0 = 0.7$, $E = 19$, and $k_{20} = 0.001$ and (B) after minimizing the sum of squares of the residuals with the nonlinear least-squares routine Solver.

Nstep2 in order to see whether this result needs refinement. Note that such refinement only concerns the computation, and yields an estimate of the random errors (as a standard deviation) but does not answer questions such as whether, for example, the temperature in the reaction vessel was uniform, or whether other systematic errors might have been involved.

Madsen et al. provide a comparison with literature data. For ease of comparison, we multiply our final value in Table 4 for k_{20} by 60 min h^{-1} and divide by $[OH^-] = 0.1$ mol L^{-1} as appropriate, and made similar adjustments in some of the other reported data.

■ A THIRD EXAMPLE

As our final exampl,e we will use the data set reported by Hempenstall et al.[8] who used the nonisothermal derivative approach to study the stability of a solution of potassium phenoxymethylpenicillin buffered at pH 9, and published one of their data sets. Use of LSPoly1 shows that its temperature can be fitted quite well by a relatively low-order polynomial in time, as illustrated in Figure 8, as long as the first data point is excluded, as was also done by Hempenstall et al. With that polynomial, we then generated a table of temperatures at 1-min intervals, and subsequently interpolated it using smaller time intervals with the slightly adjusted version Nstep3, which uses a fourth-order polynomial to compute the temperature as a function of time, and is based directly on the Arrhenius eq 4.

We find $c_0/C = 0.9996_2 \pm 0.0007_0$ (which is not statistically different from 1), $E = 91.5_0 \pm 0.2_4$) kJ mol^{-1}, and $k' = (2.9_3 \pm 0.2_6) \times 10^{12}$ min^{-1}. For this particular data set, Hempenstall et al. reported $E = 91.3$ kJ mol^{-1} within a range from 90.9 to 91.7 kJ mol^{-1}, and a k'-value of 2.71×10^{12} min^{-1} within the range

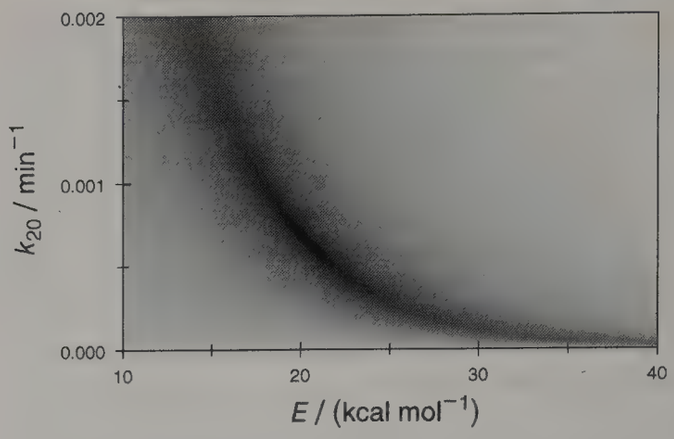

Figure 7. A gray scale map of the error surface for the model used here with the experimental data reported by Madsén et al.

Table 3. The Results of Changing the Number of Equidistant Time and Temperature Steps, N_s, within the Spreadsheet Intervals Used in Rows 40 through 340, which for $N_s = 1$ were $\Delta t = 0.5$ min and $\Delta T = 0.3701$ °C

N_s	A_{init}	E/(kcal mol^{-1})	k_{20}/(10^{-4} min^{-1})[a]
1	$0.640_4 \pm 0.001_3$	$20.2_6 \pm 0.2_3$	$3.2_3 \pm 0.1_4$
10	$0.640_4 \pm 0.001_3$	$20.2_8 \pm 0.2_3$	$3.1_9 \pm 0.1_3$
100	$0.640_4 \pm 0.001_3$	$20.2_8 \pm 0.2_3$	$3.1_9 \pm 0.1_3$

[a] Using more steps than with $N_s = 100$ has only a minor effect on k_{20}, but increases its uncertainty (because of accumulative truncation errors) and computation time.

$(2.35-3.12) \times 10^{12}$ min^{-1}. Our single result falls well within these ranges. Moreover, Hempenstall et al. listed a number of values for three different data sets (all apparently different from the above-quoted one), for both differential and integral non-isothermal analysis, the latter using the method of Madsen et al.,[6] as well as for isothermal measurements. They reported values for E ranging from 86.5 to 94.5 kJ mol^{-1}, and for k' from $(0.43-8.41) \times 10^{12}$ min^{-1}, where both ranges again widely bracket our result.

As in examples 1 and 2, we observe a linear correlation coefficient between E and k' of near unity, in this case of 0.99989, suggesting why these numbers may vary so much depending on experimental noise in individual measurements, because collinearity makes this analysis method quite sensitive in this respect. The error surface is illustrated in Figure 9, and may appear to show a different trend because the dependence of k' on E in the "trench" is opposite from that of k_{20} or k_{25} on E. This paradox indeed disappears when we instead compute and plot k_{20} or k_{25} versus E.

■ DISCUSSION

In this article, we have emphasized the spreadsheet layout more than the details of the specific software used, because of the visual nature of the spreadsheet and the importance of a transparent data organization. The software used has been described earlier,[10] is freely available for noncommercial use,[12] and is self-documented and open-access, which means that it can be examined in detail by anyone so inclined, can be modified to adapt to specific needs, and can even be pilfered for useful parts.

We have illustrated a fast, efficient, quite general, and readily accessible method to estimate the kinetic temperature stability of

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Table 4. Comparison of Reported Rate Parameters for the Base-Catalyzed Degradation of Riboflavin

$k_{20}/[OH^-]$/ (L mol^{-1} h^{-1})	E/ (kcal mol^{-1})	Authors	Ref	Comments
0.18	17.85	Rogers	2	*ni**
0.16	19.2	Guttmann	17	*i**
0.14 ± 0.02	20.39 ± 0.05	Madsen et al.	6	*ni*, integration method
0.22 ± 0.005	20.12 ± 0.04	Madsen et al.	6	*ni*, integration method*
0.192 ± 0.008	20.3 ± 0.1	this analysis		*ni*, numerical simulation

i denotes isothermal, ni nonisothermal. Base used 0.1 M (where indicated with *) or 0.05 M NaOH.

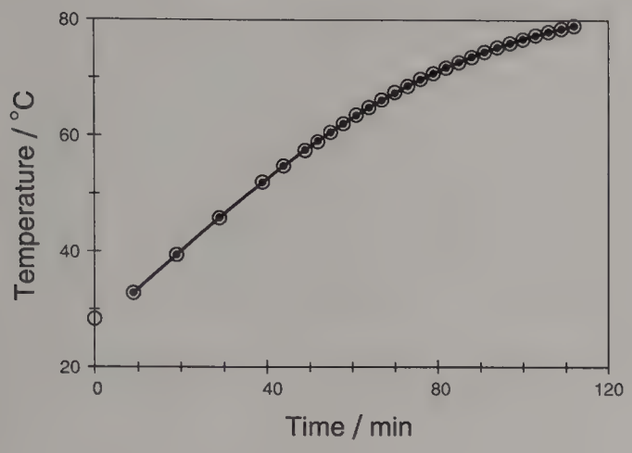

Figure 8. The temperature–time profile used by Hempenstall et al.[8] (open circles), and a fourth-order power series with $a_0 = 26.8_8 \pm 0.1_1$, $a_1 = 0.65_0 \pm 0.01_1$, $a_2 = (1.1_9 \pm 0.3_4) \times 10^{-3}$, $a_3 = (-4.1_7 \pm 0.4_0) \times 10^{-5}$, and $a_4 = (1.4_6 \pm 0.1_6) \times 10^{-7}$ fitted to these data (small solid points connected by thin line).

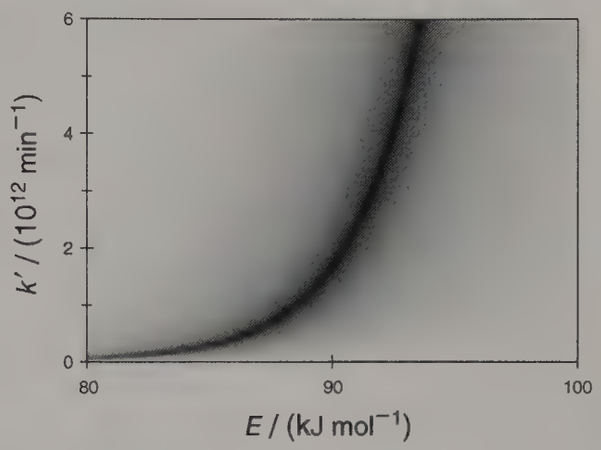

Figure 9. The error surface for our analysis of the penicillin data of Hempenstall et al.

solutions of biological or medicinal importance. Already in 1976, Kulshreshtha[18] listed well over 400 publications in this field, which has rapidly expanded since that time. The method proposed here is fast, readily adaptable to any reaction mechanism or combination thereof, of any (integral or other) order, can be used with any temperature–time profile, is conceptually simple and easily implemented, has built-in checks for numerical accuracy, and provides estimates of the precision of its results. It is described here mainly to illustrate the general power and convenience of *numerical simulation coupled with nonlinear least-squares analysis*. It can of course be implemented in any numerical software system, and is here illustrated with Excel, at present the most ubiquitous numerical software available. When used routinely, it might be worthwhile to use a more accurate fourth-order Runge–Kutta algorithm instead of the semiexplicit Euler method used here for reader transparency, in which case we may not even need a custom function such as NStep.

In the above examples, we have assumed first-order kinetics because these were known to be applicable in these cases, and therefore sufficed to demonstrate that the proposed method works. Moreover, the method will often yield better or worse fits depending on the appropriateness of the kinetic model used, but that may not always be a reliable criterion. When the kinetics are unknown, users would therefore do well to perform preliminary isothermal experiments to establish the type of kinetic model applicable to their system before attempting a nonisothermal approach.

Some problems inherent in experimental studies of chemical kinetics, such as the collinearity illustrated in the companion publication (DOI: 10.1021/ed100947d),[1] cannot be avoided, but these do not appear to present any serious impediments in the cases shown here. The use of nonlinear least squares avoids the need for special derivations, and circumnavigates the constraints posed by linear least-squares methods. The present approach is also sufficiently transparent to be practicable in the undergraduate physical chemistry laboratory. In general, one can do far more with the combination of numerical simulation and nonlinear least-squares than one can ever hope to achieve with closed-form solutions and linear least squares, and future chemists should be exposed to these easily applied yet very powerful data analysis tools, which are unlikely to disappear any time soon, but instead are certain to evolve further, because the combination of simulation and nonlinear least squares can form an intuitive, highly flexible, readily available yet very potent *general approach to data analysis*, especially now that simple visual tools are available to guard against local minima.[1]

■ ASSOCIATED CONTENT

Supporting Information

Appendix showing how to use a simple custom function. This material is available via the Internet at http://pubs.acs.org, and in ref 12.

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First-Year University Chemistry Textbooks' Misrepresentation of Gibbs Energy

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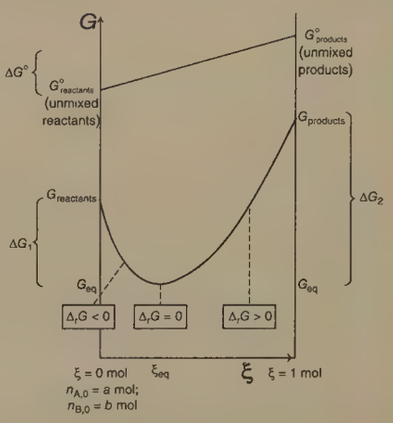
ABSTRACT: This study analyzes the misrepresentation of Gibbs energy by college chemistry textbooks. The article reports the way first-year university chemistry textbooks handle the concepts of spontaneity and equilibrium. Problems with terminology are found; confusion arises in the meaning given to ΔG, $\Delta_r G$, ΔG°, and $\Delta_r G^\circ$, which results in many textbooks not differentiating between ΔG and $\Delta_r G$. Also, there is confusion over when standard conditions apply and when they do not. A problem with the proper use of units is also found. Finally, it is suggested that most of these difficult concepts could be removed from the first-year university chemistry syllabus because (i) an accurate presentation of Gibbs energy would be far beyond an introductory chemistry level and (ii) current attempts to introduce those difficult concepts in first-year university chemistry courses are usually full of misleading formulations.

KEYWORDS: First-Year Undergraduate/General, Curriculum, Physical Chemistry, Misconceptions/Discrepant Events, Textbooks/Reference Books, Equilibrium, Thermodynamics



Research on learning difficulties associated with thermodynamics is well documented. These studies have characterized student conceptions of energy,[1] phase changes,[2,3] equilibrium,[4−7] and the second law of thermodynamics.[8−10] Comprehensive reviews covering students' conceptual difficulties about several thermodynamic ideas such as chemical equilibrium,[7] chemical energetics, chemical thermodynamics,[1] and entropy[11] establish that students have significant learning difficulties with thermodynamics.

Research studies[12,13] suggest that one of the sources of the students' learning difficulties in physical chemistry lies in how textbooks and teachers deal with key chemistry concepts. For example, several authors[4,14−20] have made an inventory of university students' misconceptions due to a poor understanding of the spontaneity concept. Some of those misunderstandings may have their origin in the way this concept is taught.

Project 2061's *Benchmarks for Science Literacy*[21] and the *National Standards for Science Education*[22] called for the inclusion in textbooks of terms meaningfully defined and of appropriate representation of key ideas.[23] These documents recognized the importance of textbooks and their evaluation. Thus, evaluation of science textbooks has become an important area of research and the inconsistencies in presentation of the subjects among textbooks are a major concern for both teachers and learners.

In a recent study,[24] it was found that chemistry textbooks often do not explicitly distinguish between thermodynamic (K) and practical equilibrium constants (K_c and K_p). For example, in many cases, K_c (or K_p), instead of K, were used to calculate $\Delta_r G^\circ$ and $\Delta_r G$. Thus, it was asserted that students could not be introduced appropriately to Gibbs energy. Hence, this work is aimed at analyzing if Gibbs energy is misrepresented by college chemistry textbooks.

This article deals with the concepts of spontaneity and equilibrium. First, a thermodynamic discussion will allow us to differentiate some important thermodynamic quantities: ΔG, ΔG°, $\Delta_r G$, and $\Delta_r G^\circ$. The different meanings of these quantities will be discussed with the help of one figure. We will focus mainly on discussing the meaning of spontaneity; that is, this concept refers both to determining whether a reaction is product or reactant favored and to predicting the direction in which a reacting system shifts in response to a disturbance. This foundation addresses some current misrepresentations. Finally, keeping this analysis in mind, we will study how general chemistry textbooks deal with all these related concepts and report some of the possible sources of misleading thermodynamic treatments.

Although we are going to deal with some advanced thermodynamic concepts, the main purpose of this study is not to provide a full background in these concepts. Nonetheless, our review of prior work in the aforementioned areas may be useful for those who need an extended and more detailed mathematical or conceptual approach.

METHODOLOGY

The analysis of textbooks has involved a qualitative approach for achieving the aim described above. For this purpose, 30 first-year university chemistry textbooks[25−54] have been analyzed. These texts are well-known first-year chemistry textbooks that have gone through several editions, thereby showing their acceptance by chemistry teachers. Although the textbooks were originally written in English and their authors are mainly from United States and Great Britain, most of them are found on the shelves of the libraries of many chemistry colleges in countries where English is not the first language. They are (or were) usually recommended to first-year university chemistry students, and most of them have been translated into several languages. Also,

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Table 1. Summary of Both Spontaneous and Equilibrium Conditions in Usual Chemical Reaction Systems

Constant variables	T, V	T, p
Spontaneity condition	$dA < 0$	$dG < 0$
Equilibrium condition	$dA = 0$	$dG = 0$

Table 2. Values of $\Delta_r G$ and Their Meaning $[aA(g) + bB(g) \rightleftarrows rR(g) + sS(g)]$

$\Delta_r G$	$d\xi$	Spontaneous Reaction
< 0	> 0	forward
> 0	< 0	backward
$= 0$	$= 0$	equilibrium

various studies published in science education journals have included these textbooks. The total number includes recent textbooks (16) as well as textbooks published before 1997 (14 textbooks that were published between 1971 and 1996). This selection will reveal if there is any difference over time in the way chemistry textbooks deal with Gibbs energy.

■ SPONTANEITY, EQUILIBRIUM, AND THE MEANING OF ΔG, $\Delta_R G$, $\Delta G°$, AND $\Delta_R G°$

To ground our textbook study, first the different meanings of ΔG, $\Delta_r G$, $\Delta G°$, and $\Delta_r G°$ need to be explained. Doing so involves the presentation of two thermodynamic energies: Gibbs (G) and Helmholtz (A). The fundamentals of this introduction are usually developed with more detail in physical chemistry textbooks,[55−59] as well as in advanced thermodynamics textbooks.[60−63] Ultimately, those current approaches are rooted on the modern thermodynamic definition of *affinity* due to de Donder.[64] That is, the fundamentals of Gibbs energy[65−67] will serve as a basis for discussion. Eventually, this previous analysis will help when we later review how first-year university chemistry textbooks deal with both equilibrium and spontaneous reactions.

The change in the Gibbs energy (G) at constant pressure and temperature for an ideal gas chemical reaction (i.e., spontaneous process) is presented. This procedure may also be applied to the thermodynamic energy A (i.e., chemical reaction at constant T and V). The development and integration of these mathematical treatments lead to the determination of the general condition for spontaneity. Keeping in mind this general condition, we will be able to account for the different meanings of ΔG, $\Delta_r G$, $\Delta G°$, and $\Delta_r G°$.[68] All this previous discussion will serve as a proper reference when we analyze how first-year textbooks define and use the aforementioned Gibbs quantities.

The change in the Gibbs energy (dG) is given by

$$dG = -SdT + VdP + \Delta_r G d\xi \quad (1)$$

Similarly, the change in the Helmholtz energy (dA) is as follows

$$dA = -SdT - PdV + \Delta_r G d\xi \quad (2)$$

where $\Delta_r G = \sum_i \nu_i \mu_i$ is the so-called free energy of reaction, and represents the rate of change of G with respect to the advancement of reaction (ξ), at constant T and p, and also the rate of change of A with respect to the advancement of reaction, at

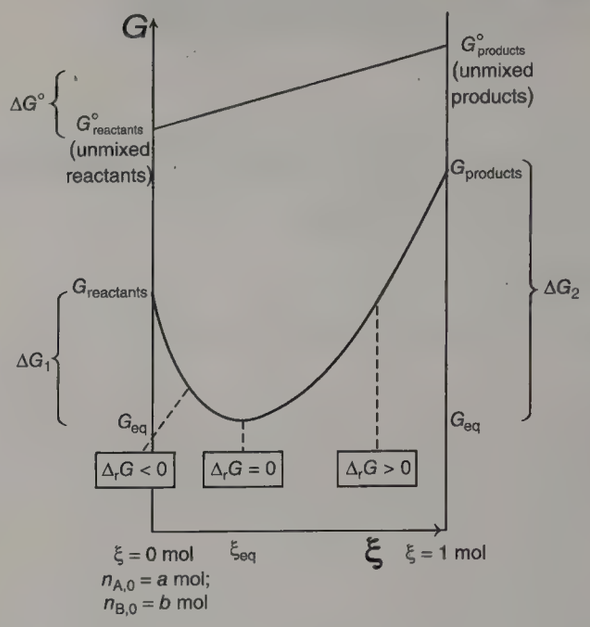

Figure 1. Variation of G as a function of ξ in a chemical process, at constant p and T. The determination of the sign of $(\partial G/\partial \xi)_{T,p} = \Delta_r G$ provides the condition of the direction of the spontaneous reaction (if $\Delta_r G < 0$: reactants → products; if $\Delta_r G > 0$: products → reactants). Furthermore, the equilibrium condition corresponds to $(\partial G/\partial \xi)_{T,p} = \Delta_r G = 0$ (minimum value of G). This way, the minimum value of Gibbs energy (G_{eq}) and vanishing $\Delta_r G$ are approached from either direction. ΔG is a finite difference; two cases are illustrated: $\Delta G_1 = G_{eq} - G_{reactants}$ and $\Delta G_2 = G_{eq} - G_{products}$. But, $\Delta_r G$ is not a finite difference: it is the rate of change of Gibbs energy with respect to the advancement of reaction.

constant T and V,

$$\Delta_r G = \left(\frac{\partial G}{\partial \xi}\right)_{T,p} = \left(\frac{\partial A}{\partial \xi}\right)_{T,V} \quad (3)$$

The meaning of this equation must be emphasized: $\Delta_r G$ is a derivative and not an ordinary difference despite the use of "Δ", as signaled by the subscript r feature.

If there is a proper control of the variables involved, the conditions that the second law establishes for both spontaneous processes and chemical equilibrium (Table 1) can be obtained. Furthermore, as

$$(dG)_{p,T} = (dA)_{T,V} = \Delta_r G d\xi \quad (4)$$

a general equation that embodies the two conditions for spontaneity outlined in Table 1 can be obtained:

$$\Delta_r G d\xi < 0 \quad (5)$$

That is, for a spontaneous reaction from left to right $[aA(g) + bB(g) \rightarrow rR(g) + sS(g)]$, $dG_{p,T} < 0$ [or $dA_{T,V} < 0$], and because $d\xi > 0$, then $\Delta_r G < 0$. For the reaction to reverse spontaneously $[aA(g) + bB(g) \leftarrow rR(g) + sS(g)]$, $dG_{p,T} < 0$ [or $dA_{T,V} < 0$], and because $d\xi < 0$, then $\Delta_r G > 0$. Thus, the sign of $\Delta_r G$ predicts the direction of the spontaneous chemical reaction.

Similarly, the general equilibrium condition can finally be written as follows

$$\Delta_r G d\xi = 0 \quad (6)$$

That is, if $\Delta_r G = 0$, equilibrium has been attained. The general conditions of forward and backward reaction, as well as that of equilibrium, are summarized in Table 2. It must be stressed that

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those conditions are not restricted to reactions at constant p and T, for they apply also to systems at constant V and T.

Keeping in mind the main purpose, the discussion will exclusively be concentrated on Gibbs energy (G). The variation of G as a function of ξ in an ideal gas chemical process is shown in Figure 1. In this particular case, it is assumed that $G^\circ_{\text{products}} - G^\circ_{\text{reactants}} > 0$. When starting with unmixed reactants in their standard states at the same temperature T and there is no mixing of reactants with products, the Gibbs energy changes linearly as the reaction progresses, and eventually the reactants are completely converted to unmixed products in their standard states at the same temperature T. But when reactants are mixed, their pressures drop below their standard state values and thus there is a drop in their Gibbs energy values ($G_{\text{reactants}} < G^\circ_{\text{reactants}}$). As the reaction proceeds, the change in the Gibbs energy is not linear. The discussion is going to focus mainly on the meaning of the curve obtained in this last case.

These valley graphs can help to clarify the different meanings of ΔG, $\Delta_r G$, ΔG°, and $\Delta_r G^\circ$.[5,63,69,70] Although modern physical chemistry textbooks usually draw and discuss the variation of G as a function of the extent of reaction, the corresponding figures are much less detailed, for their authors tend only to concentrate on the meaning of $\Delta_r G$. Hence, each of the different quantities represented in Figure 1 will be discussed.

When a reaction takes place at constant p and T, the Gibbs energy decreases, and the reaction continues until G has reached a minimum value (G_{eq}). Moreover, keeping in mind that

$$\Delta_r G = \left(\frac{\partial G}{\partial \xi}\right)_{T,p} = \sum_i \nu_i \mu_i \tag{7}$$

and recalling that the chemical potentials (μ_i) depend on the composition, we can conclude that as the chemical reaction proceeds the values of the chemical potentials change and, therefore, so does the slope of G, viz. $\Delta_r G$. In this way, the different meanings of $\Delta_r G$ outlined in Table 2 can be visualized,

$$\xi < \xi_{\text{eq}} : \left(\frac{\partial G}{\partial \xi}\right)_{p,T} = \Delta_r G < 0; \text{ that is, forward reaction allowed}$$

$$\xi > \xi_{\text{eq}} : \left(\frac{\partial G}{\partial \xi}\right)_{p,T} = \Delta_r G > 0; \text{ that is, backward reaction allowed}$$

$$\xi = \xi_{\text{eq}} : \left(\frac{\partial G}{\partial \xi}\right)_{p,T} = \Delta_r G = 0; \text{ that is, equilibrium}$$

Notice that

$$\Delta G \neq \Delta G^\circ \tag{8}$$

for

$$\Delta G^\circ = G^\circ_{\text{products}} - G^\circ_{\text{reactants}} \tag{9}$$

and ΔG is a finite difference in the Gibbs energy between two states. In Figure 1, it is, for example, the finite difference in the Gibbs energy between the final state (i.e., the equilibrium mixture) and the initial one (i.e., the mixture of reactants). Thus, ΔG differs from ΔG°, which is also a finite difference, but now ΔG° is the difference in the Gibbs energies of the products and reactants when each is in its standard state.

Also, it should be noticed that $\Delta_r G \neq \Delta G$. Not only is there a conceptual distinction between these two quantities, but there is also a distinction in the units with which each quantity is measured. In Figure 1, and as has been stated above, ΔG is a finite difference. Also, one should realize that ΔG is an extensive quantity; it is expressed in energy units only, kJ. Conversely, in Figure 1, the slope of the curve at a particular value of ξ is $\Delta_r G$. That is, $\Delta_r G$ is not a finite difference: it is an instantaneous rate of change of G with respect to the degree of advancement of reaction. It is an intensive quantity and is normally reported in units of kJ/mol, where ξ has units of mol.

At equilibrium (ξ_{eq}), the rate of free energy change is zero, $\Delta_r G = 0$, but this is not the case of ΔG. It should be stressed in Figure 1 that, when the reaction has reached equilibrium, the change in the Gibbs energy has been

$$\Delta G = G_{\text{eq}} - G_{\text{reactants}} \neq 0 \tag{10}$$

Finally, let us recall the meaning of $\Delta_r G^\circ$. It is the rate of change of standard free energy, viz.

$$\Delta_r G^\circ = \left(\frac{\partial G^\circ}{\partial \xi}\right)_T = \sum \nu_i \mu_i^\circ \tag{11}$$

Therefore, it is the slope of the standard line and is constant,

$$\Delta_r G^\circ = \frac{\Delta G^\circ(\text{kJ})}{\Delta \xi(\text{mol})} = \frac{(G^\circ_{\text{products}} - G^\circ_{\text{reactants}})(\text{kJ})}{(1-0)(\text{mol})} \tag{12}$$

$\Delta_r G^\circ$ is an intensive quantity and is expressed in kJ/mol.

Neither can the negative value of $\Delta_r G^\circ$ be used as the general condition for spontaneity, nor is it true that a positive value of $\Delta_r G^\circ$ means that a chemical reaction will not proceed. As has been discussed previously, it is the sign of $\Delta_r G$ that should always be considered for that purpose. Students can be presented selected examples[71,72] in which despite $\Delta_r G^\circ > 0$, the forward reaction is spontaneous ($\Delta_r G < 0$).

A proper calculation of $\Delta_r G$ makes use of the following equations[55,63] (in which the intensive function nature of both $\Delta_r G$ and $\Delta_r G^\circ$ should not go unnoticed)

$$\Delta_r G = \Delta_r G^\circ + RT \ln Q \tag{13}$$

$$\Delta_r G^\circ = -RT \ln K \tag{14}$$

where, Q is the reaction quotient, which has the form of the equilibrium constant, K, but is not equal to the equilibrium constant [notice that when $\Delta_r G = 0$ (equilibrium), then $Q = K$],

$$Q = \frac{\left(\frac{p(\text{R})}{p^\circ}\right)^r \left(\frac{p(\text{S})}{p^\circ}\right)^s}{\left(\frac{p(\text{A})}{p^\circ}\right)^a \left(\frac{p(\text{B})}{p^\circ}\right)^b} \tag{15}$$

We must remark that only when $Q = 1$ does $\Delta_r G = \Delta_r G^\circ$. Of course, this is not the case for most chemical reactions. Thus, we must stress that in most cases the sign of $\Delta_r G^\circ$ does not serve as a criterion for the spontaneity of a chemical process. The relationship of $\Delta_r G^\circ$ with K may be used to state how far the reaction has gone before equilibrium has been attained, for K may be obtained from $\Delta_r G^\circ$

$$K = e^{-\Delta_r G^\circ/(RT)} \tag{16}$$

If $\Delta_r G^\circ < 0$, then $K > 1$ (i.e., the process is product favored). Conversely, if $\Delta_r G^\circ > 0$, then $K < 1$ (i.e., the process is reactant favored). This determination does not depend on the isothermal

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Table 3. A Simple Condition for Both Spontaneity and Equilibrium

$$a\,A(g) + bB(g) \rightleftharpoons R(g) + sS(g)$$

$\Delta_r G$	Q/K	Spontaneous Reaction
< 0	< 1 $(Q < K)$	forward
> 0	> 1 $(Q > K)$	backward
$= 0$	$= 1$ $(Q = K)$	equilibrium

conditions (p = constant or V = constant) under which K is calculated,[73,74] as suggested by Antonik.[75]

$\Delta_r G$ differs from $\Delta_r G°$, for at a given temperature the value of $\Delta_r G°$ is fixed, but the value of $\Delta_r G$ is determined by two terms: $\Delta_r G°$ and a concentration-dependent term. These two terms can be joined into only one term, which depends on the quantity Q/K. That is, making use of eqs 13 and 14, the following equation can be obtained

$$\Delta_r G = RT \ln \frac{Q}{K} \tag{17}$$

This expression allows us to calculate the value of $\Delta_r G$ and, therefore, to discuss the direction of the spontaneous reaction (Table 2). That is, the value of the quotient Q/K may be the basis of an easier condition for spontaneity (Table 3).

The limitations of Le Châtelier's principle, as well as the misconceptions students and teachers hold when trying to apply it, have received great attention in the literature.[7,12,67,76−84] $Q-K$ inequalities (Table 3) are also easy-to-apply conditions in the prediction of the evolution of disturbed equilibria. They have the advantage of having no limitations, which is a powerful alternative to Le Châtelier's qualitative rules.[12,67,81−84]

■ SPONTANEITY AND EQUILIBRIUM: TEXTBOOKS' MISREPRESENTATIONS

Authors of first-year university chemistry textbooks scarcely pay attention to the basis of the above discussion. In most of those textbooks, there is confusion in terminology, for the terms used in the treatment of Gibbs energy are usually misrepresented (Table 4), which may lead to imprecise or even incorrect conclusions. Some of these misleading statements are reported. The equilibrium condition is often defined as $\Delta G = 0$, instead of $\Delta_r G = 0$.[25,28,30,33,38,41,44−46,48,51,52,54] Freemantle[36] states that this condition is $\Delta G° = 0$. Also, the condition for spontaneity is always defined as $\Delta G < 0$ (instead of $\Delta_r G < 0$), which is usually exemplified by calculating $\Delta G°$. Thus, the discussion of spontaneous reactions is normally restricted to standard conditions, although this situation is not always stated explicitly. That is, these presentations do not stress that restriction, which often leads to the assumption that $\Delta G° < 0$ corresponds to a general condition for spontaneity: two textbooks[31,50] include a section entitled "$\Delta G°$ as a criterion for spontaneity"; conversely, it is assumed that if $\Delta G° > 0$, the forward reaction is forbidden.

Moreover, the values of $\Delta G°$ are usually reported in kJ units for calculations involving the equation $\Delta G° = -RT \ln K$.[25−28,30,32,33,38,41,49,85,86] Moore et al.[43] and Whitten et al.[40] report $\Delta G°$ in kJ in one exercise, but in the next one, it is expressed in kJ/mol. Gilbert et al.[54] calculate $\Delta G°$ in kJ, but these units change to kJ/mol when introducing the calculated value in the above equation. Reporting $\Delta_r G°$ in units of kJ is mainly due to not using correct units of R (that is, usually the incorrect units are kJ/K). All the possible sources of this error have been discussed previously at greater detail.[72] Four textbooks[36,39,46,47] report $\Delta G°$ in kJ/mol.

Table 4. Summary of General Chemistry Textbooks' Misrepresentation of Gibbs Energy

- The equilibrium condition is often defined as $\Delta G = 0$ (also, $\Delta G° = 0$), instead of $\Delta_r G = 0$.
- The condition for spontaneity is always defined as $\Delta G < 0$ (instead of $\Delta_r G < 0$), and it is usually exemplified calculating $\Delta G°$.
- In connection with the previous point, in many cases the discussion of spontaneous reactions is implicitly restricted to standard conditions. Thus, it is often assumed that $\Delta G° < 0$ corresponds to a general criterion for spontaneity.
- It is usually assumed that if $\Delta G° > 0$ the forward reaction is forbidden.
- In many cases, the value of $\Delta_r G°$ is reported in kJ in calculations involving the equation $\Delta G° = -RT \ln K$, for authors usually do not pay attention to the correct units of R (in this case, kJ K^{-1} mol^{-1}).
- $Q-K$ inequalities are normally employed to decide the direction of a disturbed equilibrium system. This discussion is usually based on the following equation $\Delta G = RT \ln(Q/K)$, instead of $\Delta_r G = RT \ln(Q/K)$. Consequently, ΔG is reported in kJ units. Once again, some authors do not pay attention to the correct units of R.

In addition, $Q-K$ inequalities are normally employed to decide the direction of a system disturbed from equilibrium. This discussion is usually based on the following equation

$$\Delta G = RT \ln \frac{Q}{K} \tag{27}$$

(instead of $\Delta_r G = RT \ln(Q/K)$). In those cases, ΔG is usually reported in kJ units,[25,29,32,35,41,42,51,87] for authors have not paid attention to the correct units of R. But, in other cases, authors[36,37,45,48] make use of ΔG having kJ/mol units. Still, Umland and Bellama[45] explain

> We have been writing J or kJ for the units of $\Delta G°$ and ΔG. ... However, $\Delta G°$ and ΔG are extensive properties and really do include units of mol^{-1} because, in thermodynamics, equations are always interpreted in terms of moles. *In calculations that involve both $\Delta G°$ or ΔG and R, the unit J/mol (or kJ/mol) must be used for $\Delta G°$ and $\Delta G'$.* (authors' emphasis)

On the other hand, authors do not enlarge this topic to cases in which Le Châtelier's principle is limited. On the contrary, it is used to demonstrate its supposed validity. For example, a $Q-K$ discussion can help when considering the limited character of that principle when predicting the evolution of a disturbed chemical equilibrium system when adding a reactant at constant p and T.[12,61,67,71,72,76,81,84]

■ CONCLUDING REMARKS AND SUGGESTIONS FOR TEACHING

The misleading assumptions reported in this study arise from the quantitative and mathematical emphasis given to the thermodynamic concepts involved, but without explaining them in a proper way. A sound qualitative discussion would help in the clarification and differentiation of ΔG, $\Delta_r G$, $\Delta G°$, and $\Delta_r G°$. This way, many authors have proposed a revision of

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the symbolism,[5,88−91] but their suggestions have not been heeded. Still, modern physical chemistry textbooks usually comply with the IUPAC recommendations.[68]

The main conclusion of this study is that in first-year chemistry textbooks ΔG, $\Delta_r G$, $\Delta G°$, and $\Delta_r G°$ are not properly defined and are only used in algorithmic calculations. Hence, this teaching approach would promote robotic learning rather than conceptual understanding and even might be the origin of students' misunderstandings. Still, this article cannot go further stating in which specific way this widespread misleading textbook approach can lead to student misconceptions, for our attempt has been to contribute to both the distinction and proper use of each of the quantities involved in the discussion of spontaneous reactions.

With respect to $\Delta_r G$ and ΔG, the confusion in textbooks arises due to the overuse of the symbol "Δ" in teaching thermodynamics in introductory university chemistry courses. That is, it is assumed that ΔG plays the role of $\Delta_r G$. Also, there is a great deal of confusion when reporting their units. Moreover, some authors state that $\Delta G° < 0$ is a general condition for spontaneity. Significant differences were not found over time in the way college chemistry textbooks deal with ΔG, $\Delta_r G$, $\Delta G°$, and $\Delta_r G°$.

The thermodynamic foundation outlined in this article goes beyond the scope required for an introductory course.[55−64] However, current college chemistry textbooks include a great amount of information on difficult thermodynamic concepts. The stress on the Gibbs energy has not been the case historically. A glimpse at chemistry textbooks published 50 years ago[92−95] reveals that authors did not include Gibbs energy. The "chemical principles" shift that occurred in the mid-1960s[96,97] and the increasing number of pages of each new textbook edition[98,99] may explain the current emphasis given to thermodynamics in first-year university textbooks.

At this point, it is interesting to note that some authors have written general chemistry textbooks[32,42] as well as physical chemistry textbooks.[55,57] It is rather surprising that the misleading assumptions reported in this study (e.g., confusing ΔG with $\Delta_r G$) are only present in introductory textbooks. Perhaps, those authors have tried to simplify the thermodynamic topics they introduce to students at the first-year level, but their attempts might have gone too far. Therefore, maybe, it would be better to remove most of those difficult topics from the first-year university syllabus, leaving them for an advanced treatment, instead of continuing to teach them using oversimplified and misleading thermodynamic statements. The accurate thermodynamic approach given by some first-year chemistry authors[53] to the concepts analyzed in this study would seem to be far beyond the level required in an introductory college chemistry course. A balanced general chemistry course should not be focused mainly on thermodynamics because there would not be enough time to develop properly all the other essential topics to be covered. Thus, general chemistry students would not be capable of understanding those difficult thermodynamics topics, in spite of being accurately treated in their textbook, because teachers could not devote the proper time to develop such a large quantity of information.

In a recent study,[24] it was suggested that first-year university students should be introduced to practical equilibrium constants only, leaving the discussion of the thermodynamic equilibrium constant (and its relationship to $\Delta_r G°$ and $\Delta_r G$) to an advanced level. This recommendation is also suggested in this study because a sound introduction to the Gibbs energy may be too difficult for first-year students to understand.

$Q-K$ inequalities (i.e., Q_c-K_c or Q_p-K_p) could be introduced at this level as a first basic criteria for spontaneity in isothermal conditions.[100] That is, one does not need thermodynamics to distinguish between Q and K for a reaction. Indeed, it is probably a mistake to wait until the discussion of thermodynamics to make this point. One merely needs to distinguish between the ratio of partial pressures or concentrations at a degree of reaction progress (Q) and the value when the system is at equilibrium (K). Eventually, this mathematical discussion could be justified in an advanced course dealing with the second law of thermodynamics.[81]

Still, defenders of the current first-year thermodymics emphasis would argue, among other things, that engineers need thermodynamics earlier than suggested in this article or that biologists could never get to Gibbsian thermodynamics; also, many teachers would support the view that directionality and spontaneity rationalized by the meaning of $Q-K$ inequalities are enriched if Gibbs energy is introduced. Therefore, they may think that Figure 1 could be used to provide beginning students with conceptual understanding that could be expanded on in later courses. Moreover, they would also add that Gibbs energy is useful in teaching electrochemical phenomena. Unfortunately, the scarcity of time at the introductory level would not allow the Gibbs energy to be taught well and thus might not favor meaningful learning. If Gibbs thermodynamics do have to remain in the general chemistry syllabus, it must be stressed to authors that any attempt to introduce them to first-year students must simplify the conceptual approach given in this study, but without continuing current textbook errors. The teaching of thermodynamics should pay careful attention to the precise meaning of the terminology involved as well as to the demanding difficulty of the concepts to be learned. Thus, the debate about what, how, and when thermodynamics should be taught is still open, challenging future research on this topic.[1]

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The Quantification of Electronegativity: Some Precursors

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ABSTRACT: This paper calls attention to the early work of the American chemists Worth Rodebush and Groves Cartledge, and their anticipations of a quantitative electronegativity scale, which predate the classic 1932 paper of Linus Pauling by several years.

KEYWORDS: History/Philosophy, Atomic Properties/Structure, Periodicity/Periodic Table

Most chemists are under the false impression that the electronegativity concept was first introduced by the American chemist, Linus Pauling, in 1932.[1] However, in a series of historical papers published in this *Journal* it was shown that both the term and the concept predate Pauling by more than a century.[2,3] These earlier 19th-century electronegativity scales were essentially qualitative in nature, and consequently a weaker claim can still be made that Pauling—if not the originator of the electronegativity concept—was at least the first to provide a fully quantified scale for its measurement. Unfortunately even this weaker claim requires substantial qualification because recent work has uncovered two earlier pre-Pauling attempts to quantify this important chemical concept: two precursors whose subsequent fates illustrate some important lessons about how one goes about successfully developing and marketing a scientific concept.

THE *V/S* SCALE OF WORTH RODEBUSH

The first and earliest of these precursors was due to the American physical chemist, Worth H. Rodebush (1887−1959), who is perhaps best known as the coauthor, along with his doctoral advisor, Wendell Latimer, of the first paper to deal with the concept of the hydrogen bond.[4] In 1925, seven years before the publication of Pauling's paper, Rodebush published an article in this *Journal* dealing with the Bohr atom and the periodic table in which he made the following passing comment:[5]

> If it might be permissible to introduce a qualitative formula into science which is rapidly becoming exact, we might represent the electronegativity as a function of V/S where V is the number of valence electrons and S the number of shells. The basis of this formula is Coulomb's law and I believe that in a few years we shall calculate the energy changes in chemical reactions by means of it.

Ignoring Rodebush's inappropriate description of his equation as "qualititative," which may have been a typographical error for "quantitative" (as an equation must necessarily be), there is little doubt that this interesting suggestion was the result of an explicit attempt on the part of Rodebush to make the electronegativity concept more rigorous, as shown by his comments in an article written for *Science Magazine* the previous year:[6]

> I had hoped that we might be able to substitute electron affinity or ionizing potential for the wretched term electronegativity, but these quantities are measured for the gaseous state and our ordinary chemical properties are concerned with the condensed phases. For instance the electron affinity of the chlorine atom is less than the ionization energy of sodium, so that a chlorine atom should never rob a sodium atom of its electrons, and yet nothing is more certain than that it does so in a solution of sodium chloride.

The historical ambiguity is, of course, that, having suggested this explicit formula for calculating electronegativity values, Rodebush apparently did nothing further with it, though it requires only about five minutes to calculate the resulting electronegativity values for the main-block elements using valence-electron and Bohr-atom shell counts readily available in 1925, as summarized in Figure 1. The resulting values show a 0.92 linear correlation coefficient with the corresponding Pauling electronegativity scale for these elements and a 0.97 correlation coefficient with the corresponding Allred−Rochow scale, results that are essentially identical with the correlation coefficients interrelating the 25 or so modern electronegativity scales.

There are, of course, problems with extending this definition to the transition metals because the valence electrons for these atoms reside in two different shells, though use of an averaged shell number would probably give consistent results. A second problem is that the Rodebush definition gives values for the posttransition elements (Zn, Cd, Hg, Ga, In, Tl) that are too low as it does not take into account the effects of the d-block and f-block insertions on the screening constants for these elements. Likewise it gives values for H and He which are far too small, though it shares this problem with the majority of modern definitions, most of which have to instead make use of the corresponding Pauling values.

Despite these problems, the history of the electronegativity concept would have been quite different if Rodebush had properly developed his suggestion. Having a complete scale in 1925 for even just the main-block elements would have been a considerable advance over what in fact actually happened. Few chemists are aware that in his original paper of 1932 Pauling provided quantitative electronegativity values for only ten nonmetallic elements. In the 1939 edition of his famous monograph, *The Nature of the Chemical Bond*, he extended his scale to 33 elements, though he never published the data or calculations on which this extension was based.[7] Not until 1960, and the

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	1	2	3	4	5	6	7	8
2	Li (0.50)	Be (1.00)	B (1.50)	C (2.00)	N (2.50)	O (3.00)	F (3.50)	Ne (4.00)
3	Na (0.33)	Mg (0.67)	Al (1.00)	Si (1.33)	P (1.67)	S (2.00)	Cl (2.33)	Ar (2.67)
4	K (0.25)	Ca (0.50)	Ga (0.75)	Ge (1.00)	As (1.25)	Se (1.50)	Br (1.75)	Kr (2.00)
5	Rb (0.20)	Sr (0.40)	In (0.60)	Sn (0.80)	Sb (1.00)	Te (1.20)	I (1.40)	Xe (1.60)
6	Cs (0.17)	Ba (0.33)	Tl (0.50)	Pb (0.67)	Bi (0.83)	Po (1.00)	At (1.17)	Rn (1.33)
7	Fr (0.14)	Ra (0.29)						

Figure 1. Rodebush V/S electronegativity values.

	1	2	3	4	5	6	7	8
2	Li (1.29)	Be (2.54)	B (3.87)	C (5.16)	N (6.71)	O (8.19)	F (10.0)	Ne (NG)
3	Na (1.02)	Mg (1.76)	Al (2.45)	Si (3.13)	P (3.83)	S (4.55)	Cl (5.20)	Ar (NG)
4	K (0.87)	Ca (1.42)	Ga (2.20)	Ge (2.74)	As (3.26)	Se (3.78)	Br (4.24)	Kr (NG)
5	Rb (0.82)	Sr (1.33)	In (1.92)	Sn (2.36)	Sb (2.84)	Te (3.27)	I (3.74)	Xe (NG)
6	Cs (0.77)	Ba (1.21)	Tl (1.78)	Pb (2.18)	Bi (2.60)	Po (3.02)	At (NG)	Rn (NG)
7	Fr (NG)	Ra (1.24)						

Figure 2. Cartledge $\phi^{0.5}$ values (NG means "not given").

publication of the third edition of his book, did a complete scale finally appear.[8]

In addition, while the Rodebush electronegativity definition is an example of what Ferreira calls a primary definition, meaning one based on fundamental atomic properties and having a clear theoretical justification, the Pauling thermochemical definition is actually an example of a secondary definition, meaning one that is based on an empirical correlation between a macroscopic property of some sort (in this case thermochemical bond energies) and electronegativity and which is, consequently, lacking a clear theoretical justification.[9]

Lastly, it is of interest to note that the Rodebush scale provides, as shown in Figure 1, an unambiguous criterion (EN > 1.00) for the zigzag line separating the metals and nonmetals commonly found in introductory textbooks, though it still begs the question of whether this line accurately represents the separation of these two classes of simple substances in the first place.

■ THE IONIC POTENTIAL SCALE OF GROVES CARTLEDGE

While the *V/S* scale of Rodebush predated the work of Pauling by seven years, the quantitative scale proposed by the American chemist Groves H. Cartledge (1891–1980) would not appear until 1928 and, thus, predated the work of Pauling by only four years. In addition, a proper of understanding of the nature and limitations of Cartledge's work requires some preliminary background.

Younger chemists are often unaware that the early decades of the 20th century saw the development of two alternative approaches to the description of bond polarity. The first of these, owing largely to the American chemist G. N. Lewis, began with an idealized covalent bond and discussed bond polarity as a deviation from this ideal which could be expressed in terms of the relative electronegativity difference between the two bonded atoms.[10] The second approach, due largely to the Polish chemist Kasimir Fajans, began with an idealized ionic bond and discussed bond polarity as a deviation from this ideal which could be expressed in terms of the polarizing ability of the cationic bonding component, on one hand, and the polarizability of the anionic bonding component, on the other.[11] The Lewis "covalent/electronegativity" model was subsequently developed by Pauling in the 1930s and became the prevailing paradigm in the United States and Great Britain, whereas, prior to the Second World War at least, the Fajans "ionic/polarization" model was the prevailing paradigm in continental Europe and Russia.

The resulting bifurcation of the literature on bond polarity has resulted in most chemists failing to recognize that the various numerical scales of cationic polarizing ability, which have been proposed over time within the context of the Fajans approach, are essentially identical to the various electronegativity scales that have been proposed over time within the context of the Lewis–Pauling approach. Most cations correspond to atomic cores, and because scales of cationic polarizing ability are intended to measure the ability of the cation to attract additional electron density, they can also serve as a crude measure of the ability of an atom's core to retain its valence electrons, as well as to attract additional electrons: in short, they can serve as a measure of an atom's electronegativity.

In 1928 Cartledge proposed a quantitative measure of cation polarizing ability that he called the "ionic potential" (φ) and which he defined as the ratio of a cation's net charge to its radius:[12]

$$\varphi = (Z/r)_{\text{cation}}$$

In subsequent papers in which he attempted to correlate various properties with the ionic potential, Cartledge came to the conclusion that the square root of the ionic potential ($\varphi^{0.5}$) was a more effective parameter.[13−15] A plot of the numerical values for $\varphi^{0.5}$ provided by Cartledge in 1928 for the main-block elements (Figure 2) versus the corresponding Pauling electronegativity values gives a linear correlation coefficient of 0.91, whereas that for the Allred–Rochow scale is 0.96. Once again these are both comparable to the correlation coefficients interrelating various modern definitions and indicate that a $\varphi^{0.5}$ scale could also have functioned as a quantitative electronegativity scale had Cartledge chosen to present it as such. Note that, like the Rodebush scale, the Cartledge scale also provides a criterion ($\varphi^{0.5} > 3.02$) for the so-called zigzag line separating the metals from the nonmetals.

Of course, neither Rodebush's electronegativity equation nor Cartledge's ionic potential had an impact comparable to Pauling's thermochemical electronegativity scale, though both had the ability to generate a complete set of quantitative electronegativity values several decades before this was finally achieved for the Pauling definition. In the case of Rodebush this negligible impact was due to the simple fact that Rodebush failed to properly develop and publicize his definition, whereas in the case of Cartledge, it illustrates the importance of selecting proper terminology and aligning oneself with the prevailing theoretical paradigm.

A similar fate befell the measure of cation polarizing ability proposed by the Hungarian chemist, Bela Lakatos, almost 30

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years after Cartledge.[16] Termed the "effective field strength" by Lakatos, it made use of effective core charges and the Slater screening constants to define the electrostatic force field around the cation:

$$F^* = Z^*e/r^2 = (Z - S)e/r^2$$

The next year the American chemists Eugene Rochow and A. Louis Allred proposed the same definition as a measure of the electronegativities of neutral atoms.[17] While the Allred–Rochow electronegativity definition is now discussed in virtually every inorganic textbook, the Lakatos cationic field strength, like the Cartledge ionic potential, has passed into virtual oblivion.

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ARTICLE

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The Atomic Mass Unit, the Avogadro Constant, and the Mole: A Way To Understanding

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ABSTRACT: Numerous articles have been published that address problems encountered in teaching basic concepts of chemistry such as the atomic mass unit, Avogadro's number, and the mole. The origin of these problems is found in the concept definitions. If these definitions are adjusted for teaching purposes, understanding could be improved. In the present article, the definitions are discussed, and the following adjustments are suggested: (i) the feature that classifies carbon-12 for the definition as the standard be its abundance, (ii) Avogadro's number should refer directly to the standard nuclide sample, (iii) the definition of the mole be based on Avogadro's number, and (iv) the term *amount of substance* be replaced by the *collection* or *quantity of microentities*. It is also proposed that the definition of the mole is first presented for nuclides and then generalized for poly-isotopic elements and chemical compounds. A possible redefinition of kilogram as a multiple of the standard nuclide mass is also briefly discussed.

KEYWORDS: First-Year Undergraduate/General, High School/Introductory Chemistry, Physical Chemistry, Public Understanding/Outreach, Misconceptions/Discrepant Events, Nomenclature/Units/Symbols, Stoichiometry

Teaching and learning basic chemical concepts of the atomic mass unit, Avogadro constant, and the mole have been perennially difficult. In their review articles in 1981 and 2002, citing over a hundred references, Dierks[1] and Furió et al.[2] concluded that the concepts are confusing. Confirmed by more recent articles, the confusion apparently has not been clarified. In 2009 Mills and Milton[3] wrote "...such concepts as the quantity 'amount of substance' and its unit 'mole'...are...the subjects of widespread misunderstanding", while in 2010 Leonard[4] remarked "...beginning chemistry students...likely to be suffering from 'Avogadro anxiety' ". Important arguments to this debate were also provided by the discussion between Freeman, Gorin, Karol, and Cvitaš reported in this *Journal* in 2003−2005[5−11] and closed by the editorial note in 2005.[12] The discussion revealed the following:

- Attempts to improve the International System of Units (SI) have a profound effect on the understanding and, consequently, on the teaching of the concepts in question.
- The main controversy is the nature of a quantity, of which the mole is a unit.
- The current definitions of the atomic mass unit and of the mole, officially adopted in 1961 and 1971, respectively, are the primary sources of confusion.

The definitions are as follows:

- Definition 1: The Dalton (Da) and the unified atomic mass unit (u) are alternative names (and symbols) for the same unit, equal to 1/12 times the mass of a free carbon-12 atom, at rest and in its ground state.[13]
- Definition 2: Mole is the amount of substance of the system that contains as many elementary entities as there are atoms in 0.012 kg of carbon-12. When the mole is used, the elementary entities must be specified and may be atoms, molecules, ions, electrons, other particles, or specified groups of such particles (ref 13, p 115).

Further, in 1993, the IUPAC Commission on Physicochemical Terminology recognized the term *chemical amount* as the valid synonym of *amount of substance*.[6] In 2006 (ref 13, p 115) it was approved that the *substance* in the latter term can be specified. For instance, one can talk of the *amount of benzene* (preferably C_6H_6). The term *chemical amount* was not considered in the same document. To make the concepts easier to comprehend, the currently used definitions of the atomic mass unit, the mole, and the Avogadro constant will be adjusted for teaching purposes only. Ideas, concepts, and arguments extracted from numerous relevant references will be presented, whereas references aiming at improving the SI system will be taken into account only when necessary. The article is divided into two parts. The simpler case of nuclides will be considered first.

THE ATOMIC MASS UNIT

The concept of atoms and molecules was conclusively accepted in the 20th century. The determination of their masses relative to the macroscopic standards was more difficult than relating the masses of microscopic entities among themselves. For the latter, a perfect tool, namely, mass spectrometry, became available; however, a standard mass was needed and this was the origin of the concept of the atomic mass unit.

Definition 1 of the unified[14] atomic mass unit (u) originates from the agreement between physicists and chemists concerning the carbon-12 scale, reached in 1961.[15] However, the assignment of the atomic mass equal to 12 u to the nuclide of the mass number 12 in this definition creates a potential danger of misunderstanding. A student may think, erroneously, that the essential feature of carbon-12 is the mass equal to 12 u. This is why Feynman et al.,[16] Tro,[17] and Kotz et al.[18] defined the standard atom as having six protons and six neutrons in its nucleus. By contrast, Brescia et al.[19] referred to carbon-12 as "the most common carbon isotope" and Gorin,[6] as "predominant carbon

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isotope". The isotopic abundance is seen by chemists simply as an intensity of mass spectrometry peaks, and this is why I propose that the atomic mass unit is defined as exactly 1/12 of the mass of the atom of the most abundant carbon isotope. Linking the unit u with mass spectrometry is a common and useful practice, as shown in numerous textbooks.[17−22] This inspired me to propose the following imaginary experiment:

> Imagine a time-of-flight mass spectrometer.[19] Prepare samples of all nuclides of all elements and introduce them simultaneously to the spectrometer. The total number of nuclides in these samples is equal to r, the value of r not being essential. The recorded spectrum consists of r peaks, and for each peak the value of time t_i is known. Thus, a series of values is obtained: $t_1, t_2, ..., t_s, ..., t_r$. The kinetic energies of ions of all the nuclides inside the spectrometer are equal, hence $(r - 1)$ proportions can be written:
>
> $$\frac{m_i}{m_s} = \frac{t_s^2}{t_i^2} \quad (1)$$
>
> where $i = 1, 2, ..., r$ for $i \neq s$ and m_s is the atomic mass of the nuclide chosen as the standard.
>
> The values of m_i are unknown, but note that if the standard mass is known then the remaining masses can be calculated easily. By virtue of definition, the mass of the most abundant carbon isotope is assigned 12 u. Its peak is the greatest one among all the carbon peaks. Having this atomic mass defined, the atomic masses of the other nuclides can be calculated immediately.

■ THE MOLE AND THE AVOGADRO CONSTANT

Experimental Roots of the Mole Concept

The mole concept is useful in science for the following reasons: (i) Samples of the order of grams or milligrams are commonly used in macroscopic experiments. They contain a huge number of atoms, normally greater than 10^{20}. (ii) It is frequently convenient to use samples with given proportions of atoms or molecules. Stoichiometric calculations and preparation of solutions of given concentrations, as well as the application of the ideal gas law to characterize gas samples are examples taken from elementary chemistry.[23] In specific cases, the numbers of entities need to be equal.

The Existing Definition of the Mole and the Problem of Amount of Substance

With reference to the discussion in this *Journal* mentioned earlier,[5−12] the relations are examined between the terms seen in definition 2: *amount of substance* (AoS) and the itemized microscopic entities, namely, *atoms, molecules, ions, electrons,* and so forth. The term *substance is* applicable to characterization of elements and chemical compounds, that is, the type of matter with a uniform, well-defined composition (even at microscopic level). This enables us "to specify the number of identical, individual units that comprise it".[5] For substances, the term AoS is acceptable under the condition that the stoichiometric variability of chemical compounds can be ignored.

A mole of electrons, also named the Faraday constant, amounts to the charge of 96,485 coulombs, and it cannot be labeled *substance*. The same statement applies to *einstein,* as remarked by Karol:[7] " ...spectroscopists unit, the einstein... defined as a mole of radiation quanta ... definitely not being a substance under accepted terminology". Hence, for these two cases, the term AoS is not applicable.

The majority of matter in the universe has no definite composition. Rocks, plasma, or biomass cannot be named *substances.* Consequently, the mole concept is not applicable, for instance, to a piece of wood. This is why the term *amount of matter,* although embracing electrons and quanta as well as elements and chemical compounds, is also not an acceptable candidate for the quantity of which the mole is a unit, when applying definition 2. The amount of matter, whether measured by mass or volume, quantifies a system adequately, if the composition of the system is irrelevant.

Moreover, it seems that the synonym *chemical amount* proposed in 1993 is a step back compared to AoS. *Amount of substance* is based on the well-defined term *substance.* Besides, considerations concerning the *chemical amount* provoke associations with diffuse boundary between physics and chemistry and encourage more clever students to ask embarrassing questions, such as "Is the electron a physical or a chemical entity?" The modifications of the definitions approved in 2006, already mentioned in the introduction, make their understanding easier, but emphasize the ambiguity of definition 2.

To constructively shift this discussion, one can focus attention on the term *numerosity* as seen in the article by Price and De Bievre.[24] To conclude the discussion, it would seem sufficient to adjust the definition advocated by IUPAC (cited after Milton and Mills[25]) "Amount of substance is a quantity proportional to the number of specified elementary entities in a sample. The proportionality constant is the same for all substances, and is the reciprocal of the Avogadro constant." For the adjustment, I propose that *amount of substance* is replaced by *collection* or *quantity of microentities,* the term very similar to "a collection of a number of specified elementary entities", as seen in Leonard.[26] The exemplary adjusted definition would then be: *The collection of microentities is a quantity proportional to the number of specified microentities. The proportionality factor is the reciprocal of the Avogadro constant.*

■ AVOGADRO'S NUMBER

The essential feature of the definition 2 is its flexibility. When modified, it can be used to define Avogadro's number, N. Thus, two possibilities result. Either the definition of the mole is followed by the definition of N or vice versa, the definition of Avogadro's number is followed by the definition of the mole. In this article the latter case is chosen, that is, the definition of Avogadro's number involves the direct comparison with the carbon-12 standard (see ref 27) as it seems that this order facilitates understanding. Accordingly, definition 2 can be adjusted to be as follows:

- Definition 3: Avogadro's number is the number of atoms in 0.012 kg of carbon-12.

Then, definition 3 implies directly:

$$N = (\text{mass of the sample})/(\text{mass of the single atom})$$
$$= 12\ \text{g}/12\text{u} = 1\ \text{g/u} \quad (2)$$

Note that a measurement is a comparison with the standard and there are two standards for the measurements of mass: kilogram and the atomic mass unit. For practical purposes, the fractions of kilogram, gram, and milligram are preferred by chemists. The ratio of two standards cannot be chosen arbitrarily but must be determined experimentally. This is clearly shown by eq 2. The

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unit u must be expressed in kilograms,[13] then Avogadro's number is dimensionless.

The value of N was determined independently many times by several experimental methods over the period of about 150 years. Uncertainty of the determinations has been reduced significantly.[27] The most recent value of the redetermined Avogadro's number, published in January 2011, is $6.02214078(18) \times 10^{23}$.[28] However, in teaching, most commonly a value of 6.022×10^{23} is used.

It seems that Rutherford's method of the determination of Avogadro's number, applied in 1909 (for a description see ref 19, pp 137–139), is most suitable for teaching purposes. It is because the conceptual simplicity makes the experiment easy to grasp. In the experiment, a known number of alpha particles were emitted by radium owing to which the number of helium atoms resulting from their catching of electrons was also known. The volume of the helium was then measured yielding the number of helium moles. The resulting Avogadro's number is the ratio of the number of atoms to the number of moles.

Avogadro's number can also be determined by students in an electrolysis experiment; for a description see the article by Ceyhun and Karagölge.[29]

■ THE MOLE CONCEPT

The mole can be defined as follows:

- Definition 4: *The number of any objects equal to Avogadro's number is the mole.*

Thus, the numbers of $N = 6.022 \times 10^{23}$ atoms or molecules or ions or electrons are examples of moles. By this definition, the value of N has a unit, the reciprocal mole (mol^{-1}). This value is the Avogadro constant denoted N_A.[30] In the case of elements and chemical compounds, the quantities, of which the mole is a unit, are *collections of atoms* and *collections of molecules*, respectively. In the case of electrons, however, the quantity is *charge*, the term shorter than the *collection of electrons*. In the case of alpha particles, the quantity will be the *collection of alpha particles*. *Mole* is a term similar to *dozen* or *score*. These units are undoubtedly useful if the counted objects are identical, such as unused pencils, matches, nuclides, or electrons. A generalization of the mole concept for similar objects will be discussed in the next section.

The definition of the mole exposes the importance of the Avogadro constant. This constant is a scaling factor between atomic-scale entities, such as atoms and molecules, and a mole—the macroscopic base unit in the SI system.[31]

Consider a sample consisting of N nuclides of A with the atomic mass M_A u and another sample consisting of N nuclides of B with the atomic mass M_B u. By virtue of eq 2, the total mass of the first sample is equal to $N \times M_A$ u or M_A grams. Similarly, the mass of the second sample is equal to $N \times M_B$ u or M_B grams. The mass of a mole of atoms or molecules expressed in grams is called the molar mass. A comprehensive characterization of this important term is presented in the article by DeMeo.[32]

The considerations outlined above hint how to count atoms by weighing. To prepare a sample consisting of 1 mol of aluminum atoms (aluminum is a monoisotopic element!), the atomic mass of aluminum should be known (equal to 27 u) and a piece of aluminum wire weighing 27 g should be cut. Obviously, any fraction of mole can be prepared in this way. The idea of counting by weighing outlined above works well when all objects are identical or at least similar. Similarity means here that the value of average mass of objects is reasonable information. This remark is essential for understanding the mole concept in the case of poly-isotopic elements.

Finally, a quote by Lorimer[33] is highlighted: "...the mole is often thought of by chemists as an Avogadro's number of entities." The definition in question can be found, for instance, in one of chemistry textbooks available in Poland since 1999, written by Kluz and Łopata[34] and addressed to young school students aged 13–14 years.

■ GENERALIZATION

DeMeo[32] describes the following situation: for a poly-isotopic element E both the atomic masses of its constituent isotopes E_1, E_2, ... and their percentage abundances are known. Hence, the abundance-weighted average mass expressed in atomic units is E_{av} and consequently, the molar mass of element E is E_{av} grams. The mole of E can be considered as consisting of N identical *nonexisting* atoms with atomic mass equal to E_{av} u each. This concept is useful in stoichiometry. However, during spectrometric measurements, one should remember that a mole of atoms consists of different nuclides E_1, E_2, ... with slightly different (except hydrogen) atomic masses M_{E1}, M_{E2}, ... and sometimes extremely different abundances.

In the above situation, the mole concept has been applied to the ensemble of similar objects characterized by an average value reasonably determined. In this generalization, the word *reasonably* is pivotal. What does it really mean? The definition is difficult to propose; however, various examples may be helpful. A dozen eggs bought in a shop can be *reasonably* characterized by the average mass of an egg. Likewise, a mole of atoms of a poly-isotopic element is a *reasonable* concept. However, an average mass of a dozen animals may not be *reasonable* for instance, for a zoo manager preparing transportation of 12 different animals, such as tigers, butterflies, elephants, and parrots.

Further, an element that exists in the form of diatomic molecules is a slightly more complicated case. It is obvious that molar masses of O_2, N_2, and Cl_2 are equal to the doubled molar masses of O, N, and Cl. However, the effect of isotopic composition in this case is more pronounced than in the case of single atoms (see ref 22). Two stable isotopes of nitrogen and three stable isotopes of oxygen are known. Hence, a nitrogen molecule and an oxygen molecule can be formed from two atoms in three and six combinations, respectively. Consequently, three types of nitrogen molecules and six types of oxygen molecules exist. The molecular masses differ slightly within each group.

For chemical compounds, the mole concept is principally identical to that of polyatomic elements. If for some reason the molecular mass is unavailable, it can be calculated from the chemical formula of a compound, and then the average atomic masses of the elements are used. An example is instructive: The formula of a simple amino acid called glycine is $H_2N{-}CH_2{-}COOH$. The molecule consists of 5 hydrogen atoms, 2 carbon atoms, 2 oxygen atoms, and 1 nitrogen atom. The average atomic masses of these elements are multiplied by 5, 2, 2, and 1, respectively, and summed up. Thus, the molecular mass of glycine equal to 75.07 u is obtained. Correspondingly, a mole of glycine is 75.07 g. If we take into consideration that there are also two stable isotopes of carbon and two stable isotopes of hydrogen, we see that a huge number of glycine molecules can be distinguished. The isotope effect outlined above is observed as contributing to the broadening of vibration spectroscopy

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Table 1. Comparison of the Current and Proposed Definitions

Topic	Current Definition	Proposed Definition
Atomic mass unit	Definition 1: The Dalton (Da) and the unified atomic mass unit (u) are alternative names (and symbols) for the same unit, equal to 1/12 times the mass of a free carbon-12 atom, at rest and in its ground state.[13]	The Dalton (Da) and the unified atomic mass unit (u) are alternative names (and symbols) for the same unit, equal to 1/12 times the mass of the free atom of the most abundant carbon isotope, at rest and in its ground state.
Mole	Definition 2: Mole is the amount of substance of the system that contains as many elementary entities as there are atoms in 0.012 kg of carbon-12. When the mole is used, the elementary entities must be specified and may be atoms, molecules, ions, electrons, other particles, or specified groups of such particles.[13]	Definition 3: Avogadro's number is the number of atoms in 0.012 kg of carbon-12. Definition 4: The number of any objects equal to Avogadro's number is the mole.

bands. However, the discussion of this effect is beyond the scope of this article.

In a chemistry course, I suggest that the concepts could be taught in the following order. First, the concept of molar mass is almost obvious for nuclides, also the abundance-weighed atomic mass is an easy issue. Once familiar with these two concepts, a student will be able to understand the more difficult concept of molar mass of poly-isotopic elements and of chemical compounds. This scheme of teaching was adopted, for instance, in the textbook by Tro[17] where two separate subchapters were proposed: "Atomic Mass: The Average Mass of an Element's Atoms" and "Molar Mass: Counting Atoms by Weighing Them".

■ THE POSSIBILITY OF A NEW DEFINITION OF MOLE AND KILOGRAM

A measurement is a comparison with the standard. This is why the reliability of the standards is of pivotal significance for science. The standards should be "invariant under translation in space and time – even on an astronomical scale".[35] Similar to the successful cases of time and length,[7] attempts have been undertaken[33] to replace the mass standard—the only SI unit that is still defined by an artifact (the platinum–iridium cylinder kept at the Bureau International des Poids et Mesures in Sèvres, France). Other metallic mass standards in various countries have been compared with the Sèvres standard over the period of over 100 years, and stability of this standard has been questioned. This is why "the unit of mass needs to be redefined".[36]

A few possibilities of the replacement of the standard were discussed in the critical review article by Leonard;[4] the simplest case suitable for incorporation into an introductory chemistry course is presented. Equation 2, of key importance, can be easily transformed into

$$1\ \mathrm{kg} = 10^3 \times N \times \mathrm{u} = 10^3 \times N \times \{m_a(^{12}\mathrm{C})/12\} \qquad (3)$$

where $m_a(^{12}\mathrm{C})$ denotes the mass of carbon-12 atom. As Leonard[4] points out "any two of Avogadro's number, kilogram, and atomic mass unit (Da in the Leonard text) may be defined independently; then the third is determined by equation (3)". The kilogram and u are presently defined; thus, Avogadro's number is determined from eq 3.

Intensive work has been done to change this situation. The value of the Avogadro constant has been determined experimentally a number of times with an effort to minimize the uncertainty of determination. Once the criteria assumed for the relative standard uncertainties, as well as other criteria, are met,[37] the Conférence Generale des Poids et Mesures (CGPM)[38] will select the best estimate ($N_{A,best}$) as the value of the Avogadro constant, a value which, by definition, has no uncertainty. The approval of the $N_{A,best}$ by CGPM will imply that the kilogram is determined by eq 3 as the multiple of the mass of carbon-12 atom, because the definition of u remains unchanged.

The mole is a collection containing $N_{A,best}$ microentities.[39] This definition of the mole is similar to that proposed by Tro in his textbook:[17] "A mole is the amount of material containing 6.02214×10^{23} particles". A sequence of six digits in the Avogadro constant used in this definition is also seen in four recent values of N_A published in 2006–2011 (ref 28, Figure 5). Understandably, the value $N_{A,best}$ is expected to be more accurate. In teaching, however, a four-digit Avogadro constant, $6.022 \times 10^{23}\ \mathrm{mol}^{-1}$, is commonly used. In this context, the definition of Tro simulates a future situation when, upon the approval by CGPM, the uncertainty of the N_A value will be equal to zero. It would be beneficial if students were able to understand the way of thinking they would be facing during many years of their professional activity.

■ SUMMARIZING REMARKS

The controversial issues tackled in this article are almost invisible in the quoted chemistry textbooks. The current definition of the mole (definition 2) is typically given in brief, which is why the elementary entities are not always itemized and *amount of substance* is sometimes lacking. In fact, the latter term is frequently mentioned only marginally. The number of moles of substance is often referred to as the "amount" of the substance.[17] The same is also true of the term *chemical amount*. This is "...the formal name for the quantity for which the units are moles... However, almost universally, chemists talk informally in terms of the 'number of moles'" (ref 21, p 65).

The above situation can be accounted for by the obvious fact that authors of introductory chemistry textbooks cannot discuss the unclear matter in detail. A long list of shortcomings of the current definition of the mole was presented by Leonard in the article dated 2007, the first shortcoming in the list being: "The indirect nature of definition makes it somewhat difficult to comprehend".[26] The implications are obvious too. Teachers facing the lack of information are unable to clarify the doubts of students, as evidenced by the tests proposed, performed, and described by Furió et al.[40] Finally, the conclusion is obvious too. The source of shortcomings should be removed by adjusting the basic definitions. This will initiate a chain of corrections. For such adjustments, in the present article it is suggested that

- the description *the most abundant carbon isotope* be used for the definition of the standard nuclide,

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- the already defined Avogadro's number be used for the definition of the mole,
- in the currently accepted definition of the mole, the term *amount of substance* be replaced by the term *collection* or *quantity of microentities*.[26]

In this manner the internal inconsistency of the definition (definition 2) would be eliminated, that is, the term *amount of substance* would no longer be connected with the terms *mole of electrons* and *mole of quanta*. The current and the proposed definitions have been compared in the Table 1.

I also propose that in teaching:

- it is exposed that *Avogadro's number is equal to the quotient gram/atomic mass unit*, as suggested by Leonard[4] and DeMeo,[32]
- the difference between *Avogadro's number* and *the Avogadro constant* is discussed,
- the definition of the mole is first presented for nuclides, to be further generalized for poly-isotopic elements.

As outlined in this article, the redefinition of kilogram and the mole is a challenging metrological task. For its realization, a more exact value of Avogadro's number is needed, which will be followed by a lengthy formal procedure. The same, however, is not true of teaching. The new definition of the mole or the current one, if adjusted, can be included into elementary courses of chemistry even now.

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(30) Dimensionless numbers are used for counting particles and other objects. Avogadro's number, *N*, is the specific case. When Avogadro's number is used to define an *ensemble* or *package* of particles named *mole* then, by virtue of definition the unit "particles/mol" should be added. However, for many objects, such as atoms, molecules, alpha particles, and so forth, the unit *mole* is used, and therefore, only the dimension $mole^{-1}$ is added to the newly defined Avogadro constant.

$$N_A = \text{the Avogadro constant} = N\ \text{mol}^{-1}$$

The name of the particle, for example, *Si atom*, must be added too on the basis of the definition of the mole (see definition 2). This can be illustrated when taking into account the conversion of a number of moles (e.g., 2.1 mol of argon atoms) into a number of particles, that is, a number of argon atoms. For this purpose, a number of moles must be multiplied by the proper conversion factor.

$$2.1\ \text{moles} \times (\mathbf{6.022 \times 10^{23}}\ \text{argon atoms})/\mathbf{mole} = 1.265 \times 10^{24}\ \text{argon atoms}$$

In the above equation, the Avogadro constant is printed in bold type. The addition of the name *argon atoms* is consistent with definition 2.

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(39) For comparison, see the following text cited after ref 33: "The effect of this definition is that the mole is the amount of substance of a system that contains $6.02214179 \times 10^{23}$ specified elementary entities". Explanation of the metrological need for nine-digit Avogadro constant is beyond the scope of this article.

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ARTICLE

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Students Using a Novel Web-Based Laboratory Class Support System: A Case Study in Food Chemistry Education

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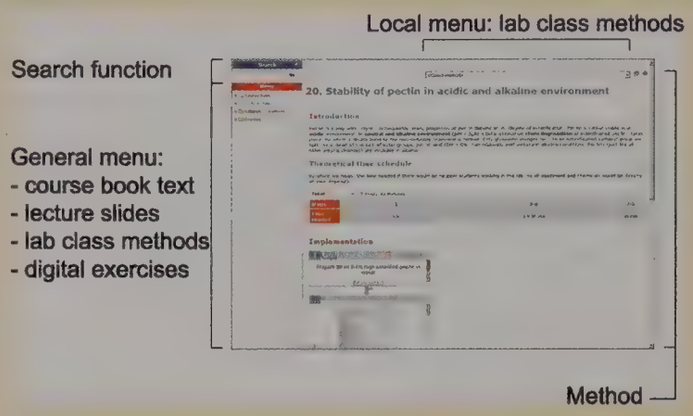
ABSTRACT: The design, usage, and evaluation of a Web-based laboratory manual (WebLM) are described. The main aim of the WebLM is to support students while working in the laboratory by providing them with just-in-time information. Design guidelines for this electronic manual were derived from literature on cognitive load and user interface design. The WebLM was introduced in a Food Chemistry course at the Wageningen University. The evaluation showed a positive attitude towards the WebLM both by students ($n = 79$) and supervisors ($n = 6$). Furthermore, the WebLM proved to be a flexible platform, easy to maintain by teachers, easy to extend for future projects, and a promising research tool for monitoring student behavior in the laboratory classes.



KEYWORDS: General Public, Laboratory Instruction, Internet/Web-Based Learning, Multimedia-Based Learning

Food chemistry education is regarded as an essential part of the academic curricula of Food Technology at Wageningen University. One characteristic of the B.Sc. food chemistry courses is that they aim to familiarize the students with a range of research methods.[1] These research methods are mainly taught during laboratory classes. In this article, we describe the design, usage, and evaluation of a novel Web-based laboratory class manual, aiming at supporting students while working in the laboratory.

THE LABORATORY CLASS OF THE FOOD CHEMISTRY COURSE

The B.Sc. Food Chemistry course (6 ECTS-credits) at Wageningen University in The Netherlands consists of two parts. In the first three weeks, students attend lectures and practice with the theory using digital exercises. The next three weeks are dedicated to a laboratory class. In this laboratory class, students work in groups of 2 to 3 students. Each group is given a raw material (e.g., barley) and investigates major chemical changes during processing (e.g., beer brewing). Students are given a list of laboratory methods and a set of assignments with which they have to make a design and schedule of their laboratory class.

From discussions with our students we know that most of them experience the laboratory class as difficult. Students have to know a large number of facts before they can make sense of what is happening during the experiments and before they can correctly interpret the results. Furthermore, students are confronted by new laboratory methods and new equipment in our laboratory class. We therefore think that the difficulties students face in our lab classes can be elucidated by focusing on the mental load of the students during laboratory work.

MENTAL LOAD IN OUR LABORATORY CLASSES

At the beginning of our research, we interviewed the most experienced laboratory supervisors ($n = 4$) of the Food Chemistry course and asked them to list the most common student questions during the laboratory class. They unanimously came up with low-level questions, such as:

- Where can I find...? Where should I put...?
- When can I do...?
- How does .... look like?

The supervisors also mentioned that higher-level questions, for example, regarding experiment design or evaluation of results, are seldom asked by students. Furthermore, supervisors mentioned that answering the low-level type of questions requires most of their supervision time.

The fact that almost no higher-level questions are being asked suggests student behavior that is characterized by Johnstone and Letton as: "[F]ollowing instructions line by line without much effort to consider the theoretical aspects which ought to illumine and inform their observations".[2] In another paper,[3] Johnstone relates students' difficulties in chemistry laboratory classes to the overloading of so-called "working memory". People have a limited working memory,[4] which can hold approximately up to 7 (±2) "chunks" of information at the same time. If a certain problem requires the learner at one time to have too many chunks of information in his or her working memory, working memory may become "overloaded" and the problem- solving process is hampered.[5] The cognitive load a problem induces is

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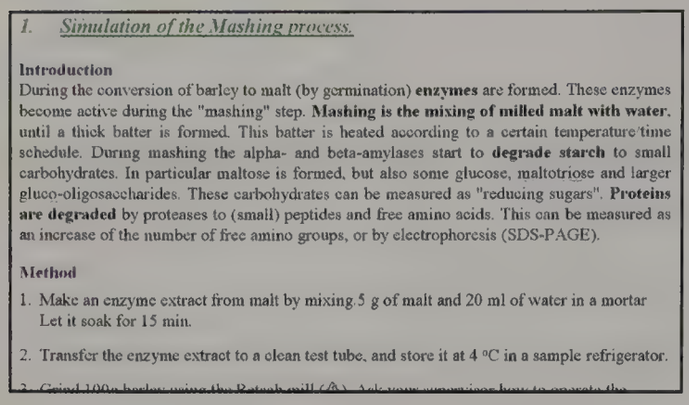

1. *Simulation of the Mashing process.*

Introduction

During the conversion of barley to malt (by germination) **enzymes** are formed. These enzymes become active during the "mashing" step. **Mashing is the mixing of milled malt with water**, until a thick batter is formed. This batter is heated according to a certain temperature/time schedule. During mashing the alpha- and beta-amylases start to **degrade starch** to small carbohydrates. In particular maltose is formed, but also some glucose, maltotriose and larger gluco-oligosaccharides. These carbohydrates can be measured as "reducing sugars". **Proteins are degraded** by proteases to (small) peptides and free amino acids. This can be measured as an increase of the number of free amino groups, or by electrophoresis (SDS-PAGE).

Method

1. Make an enzyme extract from malt by mixing 5 g of malt and 20 ml of water in a mortar. Let it soak for 15 min.
2. Transfer the enzyme extract to a clean test tube, and store it at 4 °C in a sample refrigerator.
3. Grind 100 g barley using the Retsch mill (A). Ask your supervisor how to operate the

Figure 1. A typical laboratory method, taken from the print laboratory manual of the Food Chemistry course.

Table 1. Implicit Aspects of Laboratory Method Steps

Information Students Must Know To Successfully, Safely, and Efficiently Carry Out the Experiment
Which equipment to use for common laboratory operations (e.g., one can use a beaker glass to add a liquid)
What chemicals and equipment look like
Where chemicals and equipment can be found
Hazards related to chemicals and equipment
Time needed for each method step
Whether the method step can be paused or not
How to perform the step/how to operate equipment
What the step's pitfalls are and how to avoid them
The relationship between theory and the operation in the step (the "why" of the step)

related to the problem's complexity, the learner's knowledge, or the way the problem is presented.[5,6]

■ ROLE OF THE LABORATORY MANUAL

In our laboratory classes, students perform experiments using a laboratory manual containing several methods. A typical chemistry laboratory method consists of two basic components: An introduction and a numbered list of method steps, the "recipe" (see Figure 1).

Each method step has implicit aspects required to successfully and efficiently carry out the experiment (see Table 1). These aspects are usually well-known to an expert, but unknown to students unfamiliar with the experiment. These implicit aspects can induce cognitive load.[2] Furthermore, instructional formats requiring learners to mentally combine different sources of information before understanding occurs can cause high cognitive load and (negatively) affect learning.[7,8] Lowering this type of cognitive load by integrating illustrations of the equipment in the manual text indeed resulted in improved learning outcomes.[9,10] Finally, van Merrienboer and Kirschner[11] advocate "just-in-time" provision of procedural information, namely, while they are carrying out a laboratory method.

Web-Based Laboratory Manual Might Offer Opportunities

Incorporating the implicit aspects listed in Table 1 into the printed laboratory manual might generate an increase of unnecessary cognitive load. For example, one could add a table to the printed manual, in which all materials and locations are listed, but this would lead to extensive leafing through the manual. The main advantages of Web technology over printed textbooks (see Table 2) make a Web-based version of the laboratory class manual an interesting alternative of the printed version. Such a "Web lab manual" could support students while working in the laboratory, by giving them just-in-time access to information they would otherwise have obtained from their supervisors or peers. To our knowledge, no such Web-based laboratory manual currently exists. Another opportunity of using Web technology is that it makes extensive student logging possible. Each mouse click or keyboard usage can easily be stored into a database and used for later analysis by teachers and educational researchers.

Table 2. Main Advantages of Web Text over Printed Text[a]

Compared to Printed Text Resources, Text on the Web Can:
Provide information just-in-time (and thus reduce unnecessary cognitive load)
Be relatively cheap to develop, maintain, and distribute
Provide tailored instruction, e.g., by hiding information for more experienced students
Be interactive
Provide animations/videos
Provide quick access to information (hyperlinks and search functionality)

[a] See refs 8 and 12.

■ RESEARCH AIMS

The aim of this research was to design, implement, and evaluate a Web-based laboratory manual (WebLM), dealing with the problems described before. Furthermore, the project aims to give answers to the following research questions:

1. Is it possible to design, realize, and implement a Web-based lab manual that:
   a. Students prefer to use over a printed version?
   b. Supervisors see as a valuable addition to the laboratory class?
2. Can the Web-based lab manual be used as a research tool to monitor student behavior in the laboratory class?

Design-Oriented Approach

Because it is impossible to answer the research questions above without a WebLM, a design-oriented research approach was chosen. Design-oriented research aims at the production of new knowledge by designing and realizing a new artifact.[13] To guide the design process, we adapted a design oriented research model described by Verschuren and Hartog.[14,15]

Design Assumptions and Requirements

We assumed that laboratory class teachers would not want to invest much time (maximum of 1.5 h per method) in conversion of any available printed manual into a Web-based manual. In the laboratory, there should be sufficient computers with Internet connections available on the laboratory benches. The WebLM methods are provided as standard Web pages, so they can be shown by all HTML-capable devices. Finally, the WebLM is implemented on a Web server that includes PHP and MySQL.

On the basis of the considerations mentioned above, a set of design requirements and evaluation measures of the WebLM were formulated (see Table 3).

To ensure the quality of the WebLM, design guidelines from the literature were followed during the design process:

- Use pictures when appropriate[9,16]

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Table 3. Design Requirements and Evaluation Measures for the Web Lab Manual

Item	Design Requirements—The Design Should:	Evaluation Strategies and Sources (Questions Asked of Students, Supervisor Interviews, or Monitoring of Actual Use[a])
d1	Help students while doing experiments by giving them in situ access to the WebLM	Questions concerning computer usage and student appreciation
		Measure WebLM usage
		Supervisor's opinion
d2	Be easy to use and have a clear user interface	Ask whether students find the different aspects of the WebLM clear and easy to use
		Ask and observe whether students prefer to use the printed or the e-lab manual
d3	Help students in planning their experiments	Ask whether students find the timetable/visual aids helpful
d4	Help students to work efficiently	Ask whether the WebLM saved students time/effort
		Ask and observe whether students prefer to use the printed or the e-lab manual
		Supervisor's opinion
		Count the number of times students access the "where" information
d5	Be flexible, easy to maintain everywhere and anytime	Ask whether the supervisors find the system flexible and easy to maintain

[a] Evaluation questions use a five-point Likert scale (1 = agree, 5 = disagree) for response. The design requirements are considered to be fulfilled when the average rating is 4.0 or more and at least 75% of the students rate an item as 4 or 5.

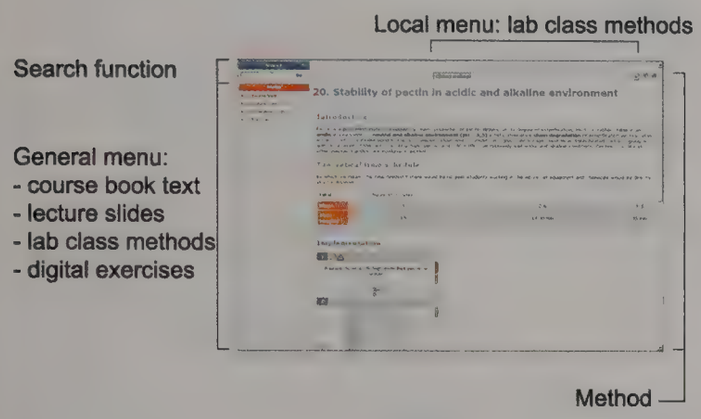

Figure 2. The overall screen layout of the electronic course book showing a laboratory method.

- Provide just-in-time procedural information[11]
- Prevent split-attention effect; improve spatial contiguity effect[5,17,18]

■ THE WEB LAB MANUAL (WebLM)

A prototype of the WebLM was realized. The WebLM's user interface is shown in Figure 2 and explained with comments in balloons as shown in Figure 3. The WebLM was incorporated in an electronic course book developed previously.[19] Because of this, students had access to other course elements, like the course book text and digital exercises. All in all, 50 laboratory methods having in total 481 method steps were added.

Bringing the Web Lab Manual to the Laboratory

Desktop computers with an Internet connection were installed on the lab benches, giving each group of 2 to 3 students access to one PC. On their PC, students had access to Microsoft Office, MSN messenger, and a Web browser. Besides the electronic version, students received a printed copy of the laboratory class manual. This printed version was similar to the version used in previous years. It contained the methods' introduction and the method steps' detailed content. Students were free to choose which manual they preferred during the laboratory class and could switch between the two versions at any time.

In total, 79 students participated in the 2008−2009 laboratory class of the Food Chemistry course. Students were distributed over 26 groups. These groups were supervised by 6 supervisors, of which 2 were new to this laboratory class.

Evaluation

Within the design-oriented approach of this project, the aim of the evaluation is to find out whether the design requirements are met.[14,15] For this, the design of the WebLM was evaluated based on

1. Logging results of WebLM usage
2. Responses from a student questionnaire ($n = 74$) held one week after the laboratory class had ended
3. Supervisor interviews held one month later ($n = 4$)

■ RESULTS

Every time the student opened a method or clicked a link or button this action was stored in the database. The logging results related to the WebLM usage are plotted against time in the figures discussed below. In Figure 4, the usage is split up per group. The figure shows considerable differences between groups: for example, during the first week, some groups used the WebLM up to four times more extensively than other groups.

In Figure 5, the usage is split up by information tab, giving an indication of the students' information demand while advancing in the laboratory class. This figure shows that students clicked relatively more "why" tabs in the last lab week, which is the week they had to hand in their report. The usage declines during the laboratory class because students start writing their report in the second and third week, and spend less time experimenting.

The results of the questions directly related to the design requirements are listed in the Table 4 (the other questions being on detailed aspects; e.g., the user interface). During the supervisor interviews, the supervisors were confronted with student questionnaire results and asked for their opinion on the WebLM in general.

In general, supervisors confirmed the picture that arises from the student questionnaire results. They did not recall any student having problems with operating the WebLM. Besides that, the supervisors are convinced that the WebLM saved them time, especially because they had to answer fewer low-level questions. This is in line with the usage logging results, showing, for example,

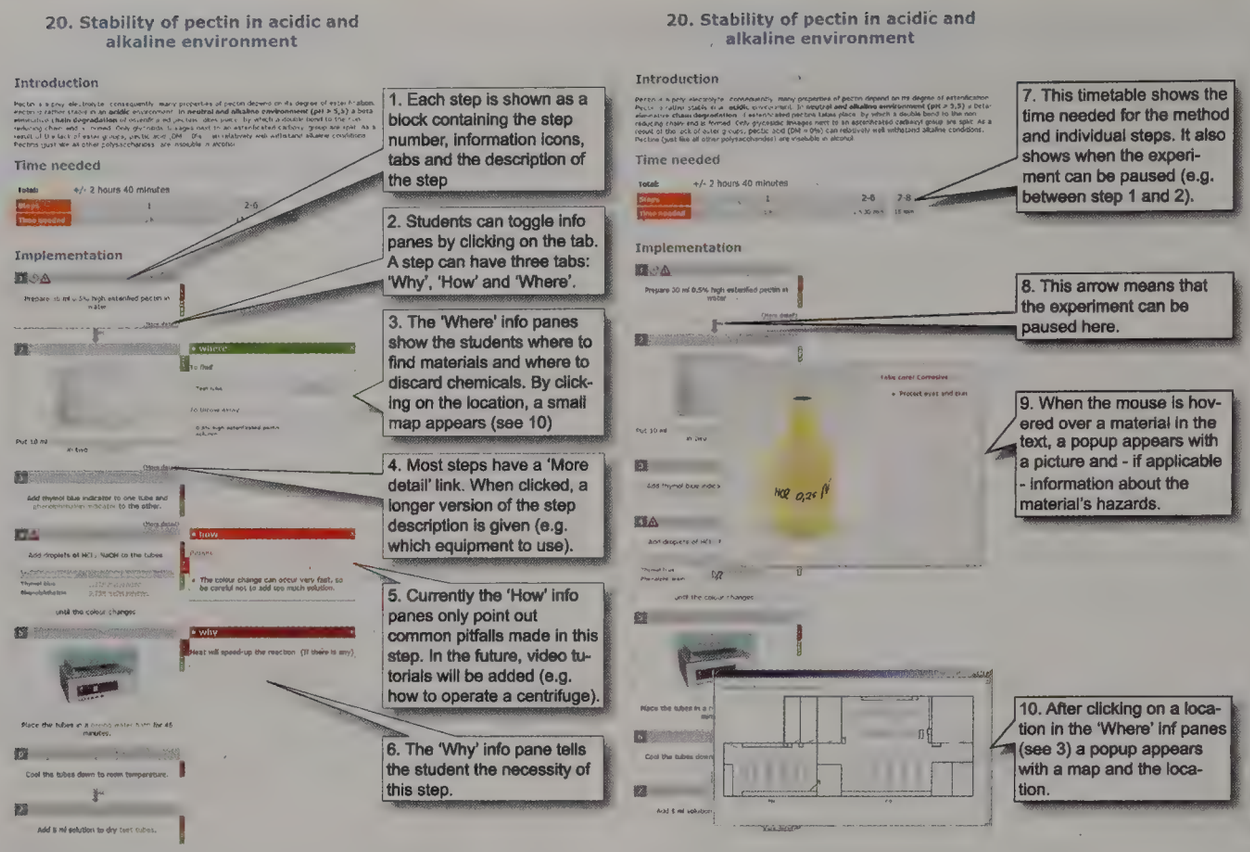

Figure 3. Detailed description of the WebLM graphical user interface, showing an almost complete laboratory method.

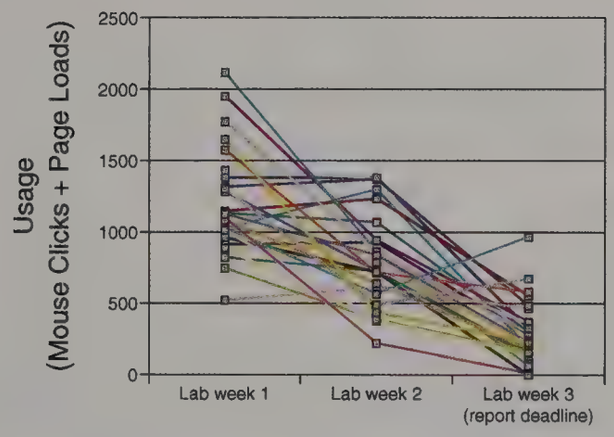

Figure 4. Number of WebLM page loads and mouse clicks during the laboratory class, the self-study, and the exam week, split up by group ($n = 26$). Each block represents a group and groups are connected with lines. This figure shows the great diversity of usage among groups, with some groups showing activity four times higher than other groups.

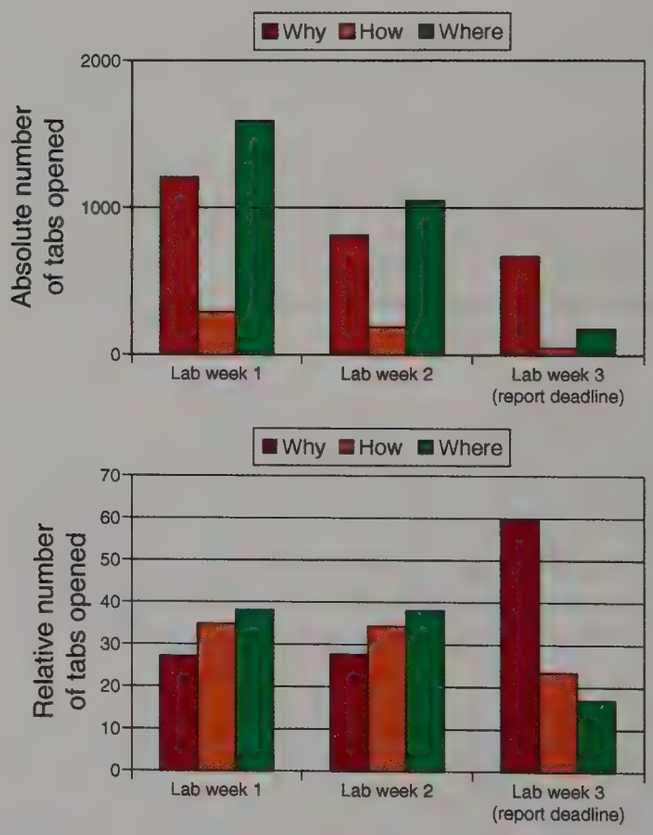

Figure 5. Absolute and relative number of times students opened an information tab during the laboratory class. To calculate the relative numbers, the absolute numbers were divided by the number of information tabs available (e.g., there are five times more "where" tabs than "how" tabs in the WebLM).

that students opened the "where" tabs more than 2800 times during the laboratory class (Figure 5).

■ DISCUSSION AND CONCLUSIONS

In this article we described the design, usage, and evaluation of a Web-based electronic laboratory manual. In this section we will discuss the results obtained using the research questions.

Is It Possible To Design, Realize, and Implement a Web-Based Lab Manual That Students Prefer To Use over a Printed Version?

Our results indicate that most students strongly prefer the Web-based lab manual over the printed version, and find the former one easy to use (q1, q4). They find the WebLM very easy to use and helpful during laboratory work (q2, q3, q5, q6, q10, q11, q12). Students think the WebLM made their lab work more

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Table 4. Distribution of Questionnaire Results

Item	Questions or Statements for Student Response	Answers (%)[a] 1	2	3	4	5	Average (SD)
q1	Which of the two versions of the lab manual did you use the most while doing experiments? (1 = electronic, 5 = printed)	49	34	7	6	4	1.8 (1.1)
q2	The WebLM helped me in preparing for an experiment.	0	11	12	46	32	4.0 (1.0)
q3	The WebLM helped me in doing the experiments.	0	1	5	34	59	4.5 (0.7)
q4	The e-lab manual is difficult to use.	77	19	3	0	1	1.3 (0.7)
q5	Because of the e-lab manual I had the feeling I knew what I was doing during the experiments.	1	4	22	56	16	3.8 (0.8)
q6	While doing an experiment, the information in the "Why" tabs helped me to understand why I was performing a step.	3	3	15	51	28	4.0 (0.9)
q7	I think I could carry out the experiments more *successfully* than with the printed manual alone.	7	8	24	28	33	3.7 (1.2)
q8	I think I could carry out the experiments in less time and with less effort than with the printed manual alone.	4	4	8	53	31	4.0 (1.0)
q9	The information in the "Where" tabs saved me time.	3	7	14	38	38	4.0 (1.0)
q10	The pictures of equipment helped me to find [them].	0	4	7	43	45	4.3 (0.8)
q11	[The timetables] helped me to plan my experiments well.	4	11	10	33	42	4.0 (1.2)
q12	I used the computer on my lab bench for:	Calculations, e.g., in Microsoft Excel (n = 46) Writing the report (n = 27) E-mail (n = 56)					

[a] Students are asked to respond using a five-point Likert scale (1 = agree, 5 = disagree) unless otherwise indicated. Shading in the Answers column represents the quantity of the responses.

efficient (it took them less time and effort to succeed in their experiments) than with the printed version alone (q7, q8, q9).

Is It Possible To Design, Realize, and Implement Web-Based Lab Manual That Supervisors See as a Valuable Addition to the Laboratory Class?

Although some supervisors had some objections to the WebLM prior to use (e.g., "students will not use it" and "I prefer the printed version"), almost all objections disappeared during the laboratory course. Nevertheless, some supervisors argued that the WebLM is not activating students to use the information offered. The WebLM offers students a great deal of information supporting students to reach the laboratory class' learning goals, but it does not always offer an incentive to make use of this information. Making the WebLM more interactive might be an interesting design challenge (see below).

One could argue that the WebLM allows students to blindly follow recipes and gather data without thinking of the purpose of the investigation. Our response to such criticism would be twofold: (i) even the most experienced chemists follow recipes, and the WebLM was designed to facilitate this part of research; and (ii) whether students think about the purpose of the investigation is not within the scope of the WebLM, but of the laboratory class as a whole.

Can the Web-Based Lab Manual Be Used as a Research Tool To Monitor Student Behavior in the Laboratory Class?

The WebLM could prove to be an interesting tool in research monitoring student behavior, because student behavior is being logged. Mining this data could result in objective information about student behavior in the laboratory class, information that otherwise could only be obtained by laborious monitoring. For example, there was great usage diversity among groups, some groups showing activity four times higher than other groups (Figure 4). It would be interesting to know how these groups performed in the laboratory class. The logging data also give clues about how many experiments a student or a group of students performs in parallel. This could be an indication of whether groups plan their work well, for example, by performing experiments during the waiting periods while other experiments are running.

■ FUTURE WORK

Now that we know that students prefer and use the Web laboratory manual, the following design challenge arises: to extend the WebLM in such a way that it trains specific cognitive skills that are often undertrained in laboratory classes. Examples of such cognitive skills include: formulating hypothesis, judging the value of experimental results, and designing experiments.[20,21] Would it be possible to develop an interactive "design layer" around the WebLM, allowing students to train their under-exposed cognitive skills while designing their laboratory class and their experiments? The problems to solve in the laboratory class would in fact be the same problems students solved in the "design layer". So both problem sets would share a lot of surface and structural features, increasing the probability of the (positive) transfer of skills.[22] In addition, this design layer would contribute to alignment between intended learning outcomes and student activities.[23]

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LABORATORY EXPERIMENT

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Determination of Fe Content of Some Food Items by Flame Atomic Absorption Spectroscopy (FAAS): A Guided-Inquiry Learning Experience in Instrumental Analysis Laboratory

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Supporting Information

ABSTRACT: This article presents a guided-inquiry (GI) hands-on determination of Fe in food samples including plantains, spinach, lima beans, oatmeal, Frosted Flakes cereal (generic), tilapia fish, and chicken using flame atomic absorption spectroscopy (FAAS). The utility of the GI experiment, which is part of an instrumental analysis laboratory course, considerably motivates underrepresented minority students, enhances student learning, and improves student critical-thinking and problem-solving ability. In addition, hands-on experience using atomic absorption spectrometry for food analysis allows students to better understand the principles and practical operation of FAAS, which they have previously learned in the instrumental analysis lecture course. The students particularly liked working as teams on their food analysis project. Furthermore, the GI food analysis experiment strategy significantly improves the overall student success rate in and enthusiasm for the instrumental analysis laboratory course, facilitating overall student success in the course.

KEYWORDS: Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning, Atomic Spectroscopy, Consumer Chemistry, Food Science, Quantitative Analysis

The employment of effective teaching strategies to improve the overall quality of education and motivate students in science, technology, and mathematics (STEM) continues to be a challenge, particularly at Historically Black Colleges and Universities (HBCU). Effective training of more minority scientists in STEM majors is of considerable national interest to promote and strengthen the future quality of our educational system and technical workforce, which is critically needed in today's global economic competition. Training scientists rather than effective technicians is important for industrial growth, breakthroughs in medical and biomedical research, and homeland security. Previous studies have demonstrated and emphasized the importance of guided-inquiry (GI) study as a more effective teaching strategy in promoting students' learning in the chemical sciences.[1−4] In addition, the utility of GI-based laboratory methodology has been shown to significantly enhance the critical-thinking and problem-solving skills of high school and college students.[5−17] Furthermore, students who participate in GI laboratory experiments typically find the lab experience more interesting, exciting, and hands-on compared to the traditional "cookbook" laboratory experiment, where students simply follow laboratory manuals.[4−16]

Consequently, an overall goal was to redesign the instrumental analysis laboratory course by incorporating the GI-based approach to enhance the basic understanding of analytical chemistry principles. To achieve this goal, an experiment on the determination of Fe content in food items was incorporated into a GI instrumental analysis laboratory course. This project was chosen because of the societal relevance and the feasibility of data collection. Iron is one of the most important and essential trace elements in the human body. It is required in diet for proper functioning of the liver and hemoglobin, a protein in humans primarily responsible for the transportation and distribution of oxygen from the lung to various human organs.[18] Iron is also a critical component of myoglobin, an oxygen-storing heme protein residing in the cell and is responsible for the color of meat.[19−22] Fe must be obtained from the diet, and foods high in iron include cereals, vegetables, milk, fish, and meat. Iron dietary supplements can also be taken daily to supply the required amount of Fe in the human body. Deficiency of Fe in the human diet, particularly in women and children, has been associated with the inhibition of hemoglobin synthesis, resulting in anemia.[23,24] Anemia may lead to serious health complications including insomnia, body weight loss, loss of strength and tiredness, decreased immune function, and breathlessness. Whereas Fe deficiency may not be prevalent in most developed countries, it has been reported to be one of the leading nutritional disorders in the world. Approximately 50% of the world's population has been diagnosed with Fe deficiency-related

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diseases.[23] Although iron deficiency in humans is often a result of a diet with low Fe content, high levels of phytates, oxalates, or phosphates in foods may inhibit Fe absorption in the human digestive system, resulting in Fe deficiency. Elevated Fe concentrations or Fe overdose has also been associated with various health problems in humans and animals.[25−28] The importance of Fe in humans and animals has generated a continued interest in the determination and monitoring of the levels of Fe in various diets. For example, the levels of Fe content of whole grains,[29] egg yolks,[30] pet diet,[31] and breakfast cereals[32−34] has been investigated in traditional laboratory experiments setting. This article describes a GI hands-on determination of Fe in some common food items in an instrumental analysis laboratory experiment.

■ EXPERIMENT

GI Experimental Lab Design

Student enrollment in instrumental analysis laboratory course, spring 2010, was made up of 11 African American students 20−33 years old, with females accounting for 55% of enrollment. Students were divided into two groups (group A and group B) to promote teamwork among the students. Each group was asked to design a sampling procedure, a preparation methodology, and an instrumental analysis technique for the determination of Fe in food samples. The lab ran for three periods of 2 h and 50 min each. Students in each group independently deliberated and selected food items to analyze. Without consultation, the two groups agreed on the use of flame atomic absorption spectroscopy (FAAS) for the determination of Fe in food samples because of its high sensitivity for metal analysis. Although the students worked as a team on their project, each student was required to submit a comprehensive individual laboratory report of their food analysis.

Chemicals and Materials and Food Sample Collection

Analytical grade and metal-free nitric acid (purity, 99.999%), Whatman (ashless) filter paper, Fe standard, and Fe hollow cathode lamp were purchased from Fisher Scientific. Group A collected plant-based food items including plantain, spinach, lima beans, oatmeal, and cereal from local grocery stores for Fe analysis. Group B collected a whole tilapia fish and whole chicken, also from local grocery stores, for Fe analysis. The collected food samples were adequately protected to eliminate sample contamination, decomposition, and to protect the integrity of the samples. The food samples were transported immediately to the laboratory for sample preparation and instrumental analysis. The whole chicken was dissected by the students in the laboratory for Fe analysis of different sections, including the chicken thigh, wing, leg, neck, liver, and heart.

Sample Preparation and Instrumental Analysis

A known mass of each food sample was digested using 6 M HNO_3 acid in a digestion flask in the hood for approximately 3 h. The digested samples were cooled to room temperature, gravity filtered using a Whatman filter paper into nitric acid precleaned volumetric standard flasks, and diluted to the mark with deionized water. The students used sample preparation and instrumental analysis procedures they learned earlier in the course. A standard Fe calibration curve was constructed by preparing six standard Fe solutions from standard Fe stock solution. The standard Fe solutions were then subjected to FAAS analysis on a spectrophotometer (Shimadzu, AA-6300) using a premixed burner air−acetylene flame. The Fe calibration curve was constructed by plotting the absorbance versus concentration for standard solutions. The high regression coefficient of the calibration curve ($r^2 = 1$), demonstrated the linearity of the calibration curve. Food samples were subjected to FAAS Fe analysis under the same experimental conditions used for the Fe standard solutions. Each sample was analyzed in triplicate and the averages of the results were reported. The constructed calibration curve was subsequently used to determine the levels of Fe in each sample.

Table 1. FAAS Instrumental Setup Parameters and Calibration Curve Equation

Parameters	Quantity
Wavelength	284.3 nm
Acetylene flow rate	15 L/min
Air flow rate	12 L/min
Band width	0.2 nm
Limit of detection (LOD)	0.20 μg/g
Limit of quantitation (LOQ)	0.65 μg/g
Fe Calibration curve equation[a]	$y = 0.0153x + 0.0011$ ($r^2 = 1$)

[a] The relationship between the absorbance and the Fe concentration is expressed as linear regression line ($y = mx + b$), where y is the absorbance measured by AAS detector, x is the Fe concentration (μg/g), m is the slope, and b is the intercept. The correlation coefficient is r.

■ HAZARDS

Students wore safety goggles and gloves at all times in the laboratory. In addition, students were told to handle concentrated nitric acid with ultimate care because it is a strong oxidizing agent and a very corrosive acid. Furthermore, the students were told not to touch the AAS flame and not to directly look at the radiation from the Fe cathode lamp during the sample analysis. All other potential laboratory hazards and safety measures including properly disposal of chicken sample waste in a designated biohazard waste container to prevent the possible spread of *Salmonella*, a common contaminant of raw poultry, were also fully discussed with the students before the commencement of the laboratory experiment.

■ RESULTS AND DISCUSSION

Level of Fe in Food

Table 1 shows the FAAS instrumental setup parameters, calibration curve equation for Fe analysis, limit of detection (LOD), and limit of quantitation (LOQ) of Fe. LOD, which is defined as the minimum detectable amount of Fe, was calculated using the equation: $LOD = (3s)/m$; where m is the slope of the calibration curve, and s is the standard deviation of the signal of the blank solution.[35] The limit of quantitation, defined as the lowest measurable concentration of Fe in food sample, was determined using the formula: $LOQ = (10s)/m$. The parameters used to calculate LOQ were as previously defined for LOD. The levels of Fe in tilapia fish and different sections of chicken are shown in Table 2. Compared to other sections, the highest level of Fe of 19.71 ± 1.50 μg/g was obtained in chicken liver. This is expected since liver is known to contain high quantities of Fe and is often recommended for anemic patients and pregnant women. The level of Fe in chicken thigh was 12.17 ± 0.22 μg/g. There was no significant difference between the levels of Fe of 5.05 ± 1.38,

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Table 2. Average Level of Fe in Fish and Chicken

Sample	Fe/(μg/g)[a]
Chicken Thigh	12.17 ± 0.22
Chicken Liver	19.71 ± 1.50
Chicken Neck	5.05 ± 1.38
Chicken Leg	5.08 ± 1.92
Chicken Wing	5.81 ± 4.29
Chicken Heart	4.41 ± 1.48
Tilapia fish	6.49 ± 4.40

[a] Standard deviation calculated from 3 samples.

Table 3. Average Level of Fe in Plant-Based Food

Sample	Fe/(μg/g)[a]
Lima beans	40.92 ± 2.62
Spinach	21.63 ± 0.34
Oatmeal	22.73 ± 4.88
Frosted Flakes cereal (generic)	16.93 ± 3.32
Plantain (inside)	7.90 ± 0.27
Plantain (skin)	5.61 ± 0.55

[a] Standard deviation calculated from 3 samples.

5.08 ± 1.92, 5.81 ± 4.29, and 4.41 ± 1.48 μg/g, obtained in chicken neck, leg, wing, and chicken heart, respectively. The Fe level obtained in tilapia was 6.49 ± 4.40 μg/g.

The results of Fe analysis in various plant-based food items are shown in Table 3. In general, high levels of Fe were obtained in lima beans, spinach, oatmeal, and Frosted Flakes cereal (generic). The levels of Fe in lima beans, spinach, oatmeal, and Frosted Flakes were 40.92 ± 2.62, 21.63 ± 0.34, 22.73 ± 4.88, and 16.93 ± 3.32 μg/g, respectively. Fe levels of 7.90 ± 0.27 and 5.61 ± 0.55 μg/g were found in plantain (inside) and plantain skin, respectively. The results of the analysis showed that the analyzed foods are rich in Fe, capable of providing the required recommended quantity of Fe needed for proper functioning of human activity.[18]

Evaluation of the Food Analysis Experiment

In addition to ensuring that the experiment honed students' analytical skills, effort was made to assess the impact of this experiment on student perceptions, attitudes, and overall success in the lab course. Students attended a discussion group with their instructor immediately after completion of the lab and a focus group with an independent evaluator at the end of the semester to discuss their experiences and perceptions of the GI laboratory experiment versus the other experiments. The students' grade distribution in the instrumental analysis laboratory course is shown in Figure 1. In general, 91% of the students earned a letter grade B or better. Specifically, 55% of the class earned letter grade A and 36% of the class earned letter grade B. Only 9% of the class earned a C letter grade. It is of interest to note that no student made D or F letter grade in the laboratory course. This is an improvement compared to grade distributions of students in previous instrumental analysis laboratory courses, demonstrating the success of GI experiments in facilitating student's learning.

The GI food analysis experiment in the instrumental analysis laboratory course was successful in producing accurate laboratory

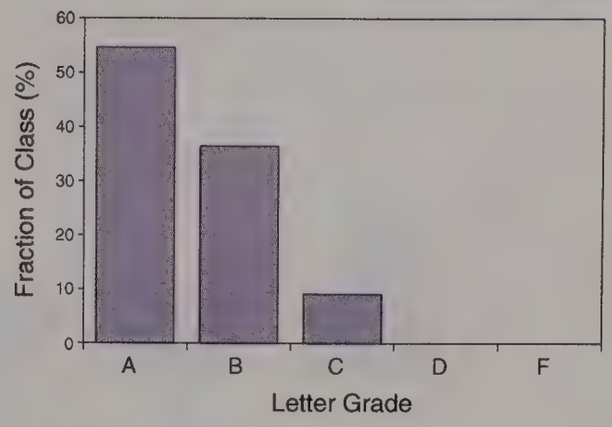

Figure 1. Grade distribution of GI students in instrumental analysis laboratory course.

analysis results. Additionally, student performance, postlaboratory discussion with students, and student surveys were used to assess its educational merit. On the basis of the postlaboratory discussions, students enjoyed the experiment and preferred its format to a traditional lab. Similar observations have been reported from previous GI laboratory experiments elsewhere.[1−17] In particular, hands-on experience using atomic absorption spectrometer was exciting and fascinating to students. In addition, hands-on experience using the atomic absorption spectrometer allowed students to better understand and relate the theoretical principles discussed in the lecture and the practical operation of atomic absorption spectroscopy. Furthermore, the experiment significantly improved the students' confidence in sample preparation, instrument handing, and made students comfortable using analytical instrumentation for chemical analysis. This is evidenced by the high square correlation coefficients of the calibration curves ($r^2 = 1$) obtained by the two groups in their Fe calibration curves and the high reproducibility (demonstrated by low standard deviation) of their triplicate sample analyses.

To further evaluate the student's perception of the GI experiment in the laboratory course, a survey based on Dalgety's Chemistry Attitudes and Experiences Questionnaire (CAEQ) and Bowen's Chemistry Laboratory Anxiety Instrument (CLAI) was administered to student participants.[36,37] Student completion of the survey was voluntary and a written informed consent was obtained from all participants. In general, the results of the survey data analysis indicate that 9 out of 11 students found the GI laboratory experiment exciting and more interesting than the traditional "cookbook" laboratory, where the students simply follow laboratory procedure. Additionally, the majority of the GI participants (9 out of 11 students) enjoyed working in teams. Working in a group setting promoted student individual responsibility because each member of the group was assigned specific duties during food sample collection, preparation, and instrumental analysis. Teamwork also facilitated and promoted individual efforts and ensured collective responsibly in a laboratory setting and allowed students to freely learn from their group members. Furthermore, teamwork encouraged constructive criticism among peers and provided motivation, ultimately allowing students to be more focused and successful in the laboratory experience. Student surveys also indicated that students in this GI laboratory course had less anxiety working with chemicals than their peers (who were also given the same survey; see the Supporting Information) and they were much more at ease recording data when compared to peers in a non-GI formatted

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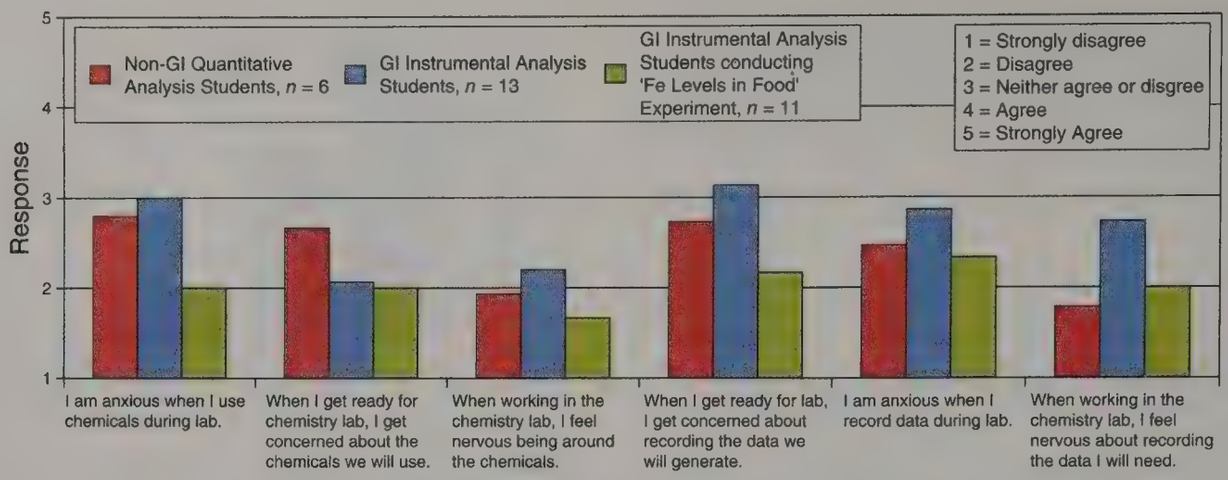

Figure 2. A comparison of survey results concerning anxiety due to using chemicals and recording data.

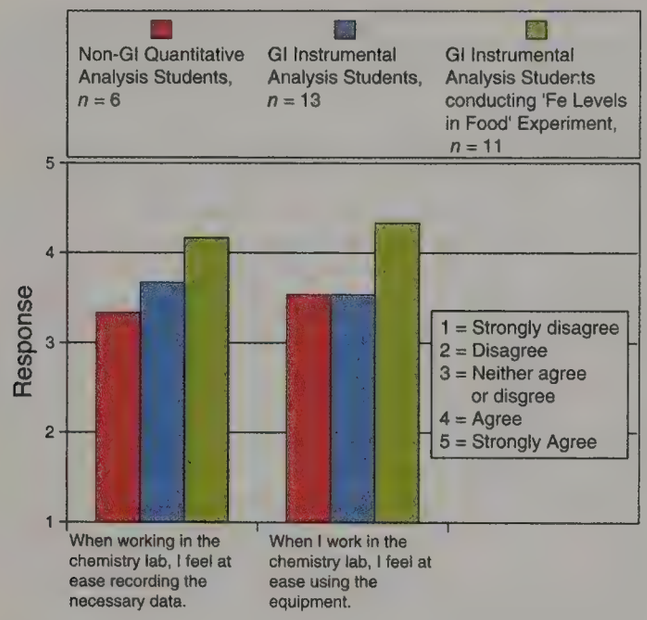

Figure 3. Comparison of survey results concerning anxiety due to recording instrumental data and using lab equipment.

analytical laboratory course and peers taking the same instrumental analysis guided-inquiry laboratory course without the addition of this food analysis lab (Figures 2 and 3). Likewise, the students who conducted the Fe in Food experiment had less anxiety toward carrying out procedures and using lab equipment. These results are similar to those seen in GI sections of other lab classes at our university.

In addition to student surveys, more open-ended feedback was received through a focus group of instrumental analysis students. Nine students from the spring 2010 section attended the focus group and all participants anticipated a career in science or medicine. Seven of the participants had independent research experience. The students identified the goals of lab in their own words as "teaching lab techniques and safety procedures". They identified the three-week GI food analysis lab as the best experience of the semester and identified it as being totally different from other laboratories. They reported being given only objectives and developing their own procedures within the assigned groups. Students particularly liked being in charge of their own experiment and enjoyed the freedom to think through most of the encountered problems and their ability to effectively resolve those problems with minimum supervision from their laboratory instructor during the food analysis. They also found that the topic, which they found very relevant, motivated them to be accurate and made their experience more memorable. The students reported that the guided-inquiry lab experience in instrumental analysis encouraged their interest in a research career because they could see the application of theories and concepts they had learned in previous classes.

In addition to practical and hands-on experience in instrumental analysis, critical-thinking, problem-solving, leadership, communication skills, and teamwork gained from the GI research laboratory experiments are highly desirable to advance and sustain the students academically and professionally in future science careers.

■ CONCLUSION

This article reports the development and introduction of a GI food analysis experiment in an instrumental analysis laboratory course. The utility of GI experiment considerably enhanced the student learning and improved the students' critical and problem-solving ability. In addition, the GI research-based laboratory strategy improved the overall pass and success rate in instrumental analysis laboratory. The students responded positively to the GI format of the experiment and particularly liked working in groups on the project. The same GI research-based teaching methodology is currently implemented in general and organic chemistry laboratories to further strengthen the undergraduate chemistry program and to improve and facilitate overall student learning.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Students notes; instructor notes; student survey. This material is available via the Internet at http://pubs.acs.org.

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LABORATORY EXPERIMENT

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Mushroom Magic: Analysis of Metals in a Familiar Food

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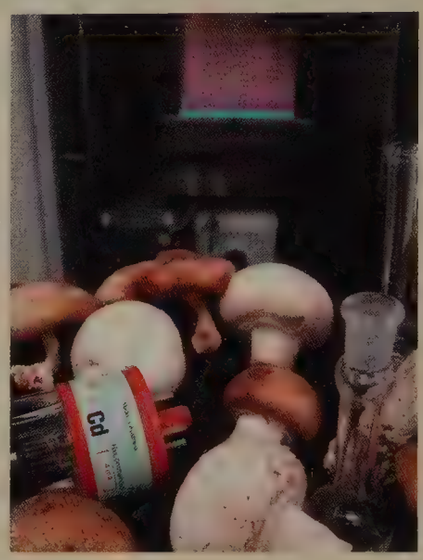
Supporting Information

ABSTRACT: Evidence suggests that student engagement in the material they are studying correlates well with better learning outcomes, and instrumental analysis modules structured to reflect student interests are of wide significance. The analysis of levels of dietary (Cu, Fe, Mn, Zn) and undesirable (Cd and Pb) metals in commercially available and wild mushrooms forms the basis for an atomic absorption experiment. The dietary metals are routinely found at levels that are easy to detect by flame atomic absorption spectroscopy, and both lead and cadmium are often found at detectable levels. Students are always more satisfied when the metals they are testing are readily quantifiable, and their "discovery" of toxic metals in a familiar food is an excellent way to promote further inquiry.

KEYWORDS: Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning, Atomic Spectroscopy, Food Science, Instrumental Methods, Metals, Nonmajor Courses



The instrumental analysis laboratory is targeted to upper-level students and one of the course goals is to help students complete the transition into independent scientists. As they progress through the semester, the students are given additional responsibility in selecting, preparing, and ultimately designing the experiments they perform. This *Journal* has helped make this transition possible by giving students ready access to a broad spectrum of well-conceived, carefully developed laboratory modules.

This approach has also afforded us the chance to discern what types of lab experiments students self-select. Often the same examples recur from year to year. When asked to choose a suitable experiment for flame atomic absorption spectroscopy (FAAS), the students repeatedly turned to experiments measuring metal(s) content in cereals,[1] multivitamins,[2] and hair.[3] Collectively, these selections and others less frequently employed[4] reflect the relevance and immediacy students feel in analyzing the components of their everyday world.

Properly performed, these experiments routinely return values consistent with the anticipated result. This makes it easier to grade the students' work, but generally fails to spark additional curiosity in the students or inspire them to probe deeper into the topic. After searching for an analyte that has multiple metals above the limits of detection, but also has the potential to be surprising, mushrooms were identified as an ideal candidate. A familiar food, mushrooms are rich in many desirable metals; however, they also contain levels of cadmium and lead that can be detected with routine flame atomic absorption methodologies. During the experiment development, a number of mushrooms were found where the lead or cadmium levels in the sample exceeded the recommended safe levels for food. Lead was quantifiable in more than 90% of the mushroom samples, and cadmium was detected in more than half. Students are almost always astonished by the levels of Pb and Cd they find, and these results are remarkably effective in prompting them to think more deeply about their data.

EXPERIMENTAL PROCEDURES

The fruiting bodies of cultivated mushrooms (white button and baby portabella varieties), purchased from our local grocery store, were divided into stems (stripes) and caps (pileus and lamella) and dried to constant mass at 70 °C in standard lab ovens. Triplicate 0.5 g samples were treated with 20 mL of concentrated nitric acid first at room temperature for several hours, then warmed slowly to 65 °C until digestion was complete and no solid material remained. After cooling, the solutions were diluted to 30.0 mL with ultrafiltered deionized (UFDI) water and capped until they were analyzed. Nitric acid blanks were prepared in the same manner. Wet digestion was the only method tested in the lab, but dry ashing[5] and microwave digestion[6] protocols are also reported to produce comparable results.

All solutions were analyzed on a Varian 240FS flame atomic absorption spectrometer, using the appropriate single-element hollow cathode lamp at its most sensitive analytic wavelength. Standard curves for each metal were prepared by diluting

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Table 1. Metal Concentrations Reported in Wild and Cultivated Mushroom Varieties

Sample Set	Pb[a]/(mg kg^{-1})	Cd[a]/(mg kg^{-1})	Zn[a]/(mg kg^{-1})	Fe[a]/(mg kg^{-1})	Mn[a]/(mg kg^{-1})	Cu[a]/(mg kg^{-1})	Ni[a]/(mg kg^{-1})	ref
14 Varieties[b] (from Turkey)	0.3–9.0	0.3–3.2	45–173	110–3640	6–197	10–89	1.2–59	6b
6 Varieties (from Turkey)	2.0–4.5	0.7–2.6	34–61	203–337	23–38	19–56	0.2–16	5b
4 Varieties (cultivated)	0.02–0.2	0.03 –1.2	47–92	28–54	5–21	5–35	N/A	8
12 Varieties (from Turkey)	0.7–4.2	0.3–54	29–146	138–1714	10–77	6–187	0.7–4.2	6c
6 Varieties (from Jordan)	2.0–4.8	0.6–1.9	36–59	212–317	24–36	19–52	0.2–13	9
2 Varieties[c] (local grocery store)	<LD–3.8	<LD–2.1	24–114	130–380	9–60	8–23	N/A	

[a] All values are reported as mg kg^{-1} of dry weight. [b] Data for *Lepista nuda* had anomalously high levels and were omitted. [c] This is student-acquired data. <LD indicates that the results were below limits of detection.

1000 mg L^{-1} commercial standards with UFDI water. More complete details regarding the instrument setup, preparation of standards, and data collection protocols are provided as Supporting Information.

■ HAZARDS

Concentrated nitric acid is extremely caustic and must be handled with care. Gases, including the toxic gas NO_2, are evolved during the digestion step, which must be conducted in a well-ventilated hood. It is important not to heat the nitric acid too soon or too fast during the digestion. In this lab, rapid gas evolution from partially digested mushrooms produced a meringue-like foamy mess. Both the analyte solutions and the standards contain dissolved heavy metals in acidic aqueous media and must be properly collected and disposed. Finally, some varieties of wild mushrooms are poisonous. If wild mushrooms are harvested for this lab, they should be treated as hazardous unless they have been positively identified as safe.

■ DISCUSSION

Mushrooms are known to be a rich dietary source of a number of metals, both essential and undesirable.[7] Results from a number of recent reports and student-generated data (Table 1) show that nontrivial levels of lead and cadmium are often detected in mushrooms. Collectively, these data demonstrate why mushrooms are an attractive choice for a student lab analyzing for metals. The levels of Zn, Fe, Mn, and Cu are easily within the limits of detection for FAAS. Additionally, many mushroom samples give detectable levels of Pb and Cd. In the experiment development, 90% of the mushrooms analyzed had measurable lead levels and approximately 50% of the mushrooms had measurable cadmium levels. The multielement nature of this sample would also make it suitable for related techniques such as ICP-MS or ICP-OES, where the quantity of detectable metals could be further expanded.[7]

There are several factors that influence the metal levels detected, including the mushroom variety and the matrix (including fertilizer choices) on which it was grown.[7] Wild mushrooms tend to have higher levels of metals than commercially cultivated samples,[7,8] with common white button mushrooms (*Agaricus bisporous*) being among the lowest.[8] The instructor can elect to influence the outcomes by judicious selection of mushroom variety;[7] however, students are most engaged in the work when they are asked to provide their own samples to analyze.

From an instructor's perspective, this lab can be used to illustrate several points. Each metal needs its own calibration curve and has its own limit of detection; having a class of students work on multiple metals clearly demonstrates that FAAS is not a monolithic technique. Also, the inherent variability in these samples is a good illustration of the need to analyze a large number of samples before trying to infer a meaningful outcome. In addition, because the lead and cadmium work requires the students to collect data near the limit of detection, the need to account for analytical blanks is critical. Almost all of the nitric acid (ACS PLUS grade) has trace levels of Cd that must be accounted for. (See Supporting Information for sample calculations, including limit of detection analysis and correcting raw data for contamination in the blanks.) Matrix interference has not been excessive, but developing calibration curves using the method of standard additions would certainly be a reasonable option.

The procedures described here are used for an upper-level instrumental analysis laboratory and require a minimum of two, 3-h lab periods to complete. Each student is expected to complete the analysis of one metal, and their resulting data are pooled to generate a complete data set. It could be compressed to a single lab period, or used in a first-year laboratory sequence, if the acidic digestion step was performed for the students prior to the start of the lab.

The initial results in this lab provide jumping off points for further student exploration. As a capstone activity in our course, students are asked to design and conduct a novel experiment based on literature methods. Since we have introduced the mushroom lab, it has been a popular source of additional projects. For example, student data collected in laboratories have suggested that lower levels of Pb and Cd were found in commercial, organic brown crimini mushrooms (also *A. bisporous*) than their nonorganic equivalents.

■ CONCLUSIONS

Mushrooms are used as experimental samples for a flame atomic absorption laboratory. They contain a number of essential metals, including Zn, Fe, Mn, and Cu, at readily detectable concentrations. Many mushrooms also contain Pb and Cd in the low ppm range. Although the Pb and Cd levels are rarely high enough to pose any true concern, students are invariably surprised to learn that a common food item could be "contaminated" in this manner. This revelation has proved effective in motivating the students to think about the results of the experiment more deeply and to do additional literature research to understand what the data really means.

■ ASSOCIATED CONTENT

Supporting Information

Student lab manual notes, including a brief background, lab instructions suitable for upper-level students, safety notes, and sample calculations; instructor notes, including an overview of

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the timeline for different aspects of the experiment, safety notes, and possible variations. This material is available via the Internet at http://pubs.acs.org.

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JOURNAL OF CHEMICAL EDUCATION

LABORATORY EXPERIMENT

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A Multicomponent UV Analysis of α- and β-Acids in Hops

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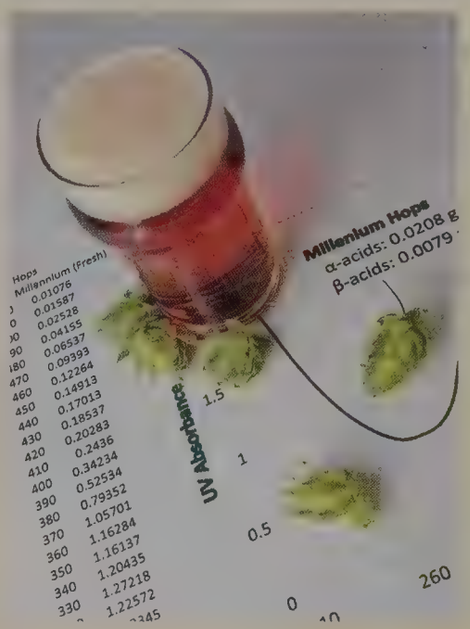
Supporting Information

ABSTRACT: A method is presented for the determination of α- and β-acids (humulones and lupulones) in a hops sample using a multicomponent UV spectroscopic analysis of a methanolic hop extract. When compared with standard methods, this lab can be considered "greener" because it uses smaller volumes of safer solvents (methanol instead of toluene). The data analysis for this lab is interesting because it relies on a three-component analysis, instead of the more common two-component analysis. This lab is educationally useful because it can be employed at any level, from a general introductory class up to an advanced instrumental class. The Supporting Information includes a lab procedure with instructor notes and an extensive math review that uses the hop system to introduce the student to Gaussian elimination, Gauss–Jordan elimination, matrix inversion, and matrix manipulation on TI calculators and in Excel.

KEYWORDS: First-Year Undergraduate/General, Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Calculator-Based Learning, Hands-On Learning/Manipulatives, Agricultural Chemistry, Food Science, Quantitative Analysis, UV–Vis Spectroscopy



This lab uses humulones (α-acids) and lupulones (β-acids) present in hops, a component of beer, to explore and explain several different chemical and mathematical concepts. The hop system is educationally engaging because it can be integrated into the curriculum at many different levels. In the nonmajors chemistry lab this experiment can be used to introduce the concept of an absolute method that does not require calibration versus a relative method that does. If a minor interfering species is ignored, the hop analysis can also be used at the general chemistry level to introduce the standard two-component analysis using high school algebra. At the chemistry major's analytical chemistry level the same lab can be used as a multicomponent analysis that includes an interfering species, and the mathematics behind the multicomponent analysis can be used as a bridge to the method of Gaussian elimination and matrix algebra to explain how systems of several equations and unknowns can be solved. At all levels a comparison of this method with the original, industry standard analysis method[1] can be used to illustrate a more "green" methodology in analytical methods development. This lab also can be used to expose students to units of specific absorptivity as well as the more often used unit of molar absorptivity. Finally, as a lab with a direct practical application to a real-world problem, a problem that will allow a student to interact with a brewer on a professional level, we predict that this exercise is one that the students will remember for years.

Most analytical chemistry texts dealing with spectroscopy discuss the Beer–Lambert law,

$$A = \varepsilon lc$$

where A is the absorbance at a particular wavelength, ε is the absorption coefficient, l is the path length, and c is the concentration for a single absorbing species. In a two-component system the absorptions of the individual components add, so

$$A = \varepsilon_{\text{comp1}} l c_{\text{comp1}} + \varepsilon_{\text{comp2}} l c_{\text{comp2}}$$

To find the concentration of both components, the absorbance of the solution at two wavelengths must be obtained, and a system of two equations and two unknowns set up.

Initially, the mixture of α- and β-acids present in a hop sample appears to be a simple two-component system that can be analyzed by standard techniques presented in most texts on UV–visible spectroscopic analysis. However, more careful analysis reveals that this system is a complex system containing at least two analytes and an additional interfering species.

Hops, the inflorescence (cone) of the *Humulus lupulus* plant, contain both α-acids (humulones) and β-acids (lupulones), the major chemical components of which are shown in Figure 1. The quantity of α- and β-acids varies with the type of hop used,[2,3] how the hop was processed,[4] how it was stored, and how long it has

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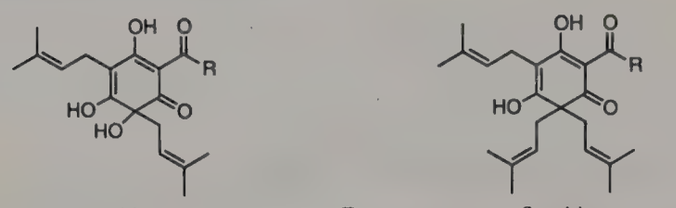

α-acids	R	β-acids
humulone	$CH_2CH(CH_3)_2$	lupulone
cohumulone	$CH(CH_3)_2$	colupulone
adhumulone	$CH(CH_3)CH_2CH_3$	adlupulone

Figure 1. Structure of major α- and β-acids found in hops.

been stored.[5] It is important for a brewer to know the quantity of α-acids in the hop used because the α-acids isomerize to form iso-α-acids during the brewing process, adding bitterness to balance the flavor of the finished beer.

In this lab students undertake a simple extraction of a hop sample followed by spectrophotometric analysis at three wavelengths to quantify the quantities of α- and β-acids as well as a third component linked to hop degradation. Upon completion of the lab the students know the percent of α- and β-acids present in their hop sample, a key number that a brewer needs to know before the brewing process has begun. The lab is closely modeled after brewing industry standards,[6] but the volume of the extract has been scaled down and an alternative extraction solvent used to make the lab more environmentally friendly. The lab is relatively short, can be easily performed in a two- or three-hour lab period, and is simple enough to be done in a nonmajors introductory class. However, depending on the mathematical sophistication of the analysis, the same data can be used in any chemistry lab from the introductory level up through instrumental analysis.

■ EXPERIMENTAL PROCEDURE

Materials

Owing to a wide interest in home brewing, a variety of hop samples can be obtained either at local breweries or on the Web, often with an estimate of the percent of α-acids the sample contains. Results obtained with two hop varieties are presented: Millennium, which typically has a high α-acid content, and Glacier, which has a low α-acid content. Commercial hop samples are already dried and can be used directly in pellet, plug, or leaf form. Fresh hop samples contain large quantities of water and must be dried before analysis; otherwise, the sample will mold quickly, even when stored at 4 °C. Drying the fresh hop sample also makes the results more consistent with dry commercial products. Hops should be stored in either a refrigerator or a freezer and be kept out of direct sunlight.

Chemicals used in this exercise include reagent grade NaOH and spectrophotometric grade methanol.

Although the results shown here display spectra obtained between 510 and 210 nm, the analysis only requires data at three key wavelengths: 355 and 325 nm, where lupulones (β-acids) and humulones (α-acids) have their maximum, and 275 nm, where both species have low absorbance. Thus, the spectroscopy can be performed on most UV−visible spectrophotometers, and the instructor has the option of having students obtain either complete spectra or data at limited wavelengths if their access to the spectrometer is limited.

The one nonstandard piece of equipment required for this lab is a grinder for grinding the hop sample. A simple household coffee grinder such as a low-end Mr. Coffee model IDS 55 gives satisfactory results for ~$20.

Procedure

Roughly 3 g of dried hop is ground to a fine powder using a household coffee grinder. About 2.5 g of this material is accurately weighed to the nearest milligram and placed in a 100 mL beaker with 50.0 mL of methanol and stirred for 30 min at room temperature. The mixture is then allowed to stand for 10 min to let the particulate matter settle, and the extract is gravity filtered to remove the particulate matter. A 50 μL aliquot of the filtrate is then placed in a 25 mL volumetric flask, and the flask is filled with methanolic NaOH (0.5 mL of 6 M NaOH in 250 mL of methanol). An aliquot of this solution is then placed in a 1 cm quartz cell, and its UV−visible spectrum is obtained using of blank of 50 μL of methanol in 25 mL of methanolic NaOH. Depending on equipment and time constraints, either a complete spectrum between 520 and 210 nm can be used or just absorbance values for the three key wavelengths of 275, 325, and 355 nm can be obtained.

Because some methanol may evaporate during the extraction process, the extraction should be performed in a closed container. For higher accuracy, the extract mixture can be weighed before and after the extraction, and additional methanol added before filtration to replace any solvent lost to evaporation.

■ HAZARDS

Methanol is highly flammable and toxic by inhalation, ingestion, or skin absorption. Waste methanol should be placed in a labeled nonhalogenated waste container for disposal by burning. Sodium hydroxide (NaOH) is corrosive and can cause severe burns.

■ RESULTS AND DISCUSSION

Extraction Rational

Petroleum ether was used for the extraction solvent in the original method describing this procedure.[1] The high volatility and flammability of this solvent precludes its use in an undergraduate lab. The industrial method presently endorsed by the American Society of Brewing Chemists[6] uses toluene instead of petroleum ether for the extraction solvent. Toluene, however, is also not a good choice for an undergraduate laboratory because it has a strong absorbance at 269 nm and slight errors in the volume of toluene used, in either the sample or the blank, can significantly affect the absorbance value at 275 nm, a key analysis wavelength. Methanol, ethanol, hexane, and ether were tried as other possible extraction solvents (data not shown). For an undergraduate lab methanol works almost as well as toluene as an extraction solvent; it has the added advantages of having negligible absorbance at 275 nm so it does not interfere with spectroscopic measurements, and it is less toxic than toluene. If this procedure is going to be used to evaluate a hop sample for brewing, however, the toluene extract should be used, because that is the industry standard.

In the standard procedure[6] 5 g of hop is extracted into 100 mL of toluene. In the procedure given here, the size of the sample and the volume of solvent were cut back to minimize expense and to make this a more "green" procedure. The lab can be made even more environmentally friendly by further reducing sample size to

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0.625 g of hops extracted into 12.5 mL of methanol, having several groups use a single extract, and by using a 20 μL aliquot of extract diluted into 10 mL of methanolic NaOH.

Typical Results

Typical results are shown in Figure 2 for three samples: a Glacier hop that is low in α-acids, a Millennium hop that is high in α-acids, and the same Millennium hop sample that has been degraded by storing at room temperature for one week. Three key wavelengths are used in this analysis: 325 nm where the α-acids have their maximum absorbance, 355 nm where β-acids have their maximum absorbance, and 275 nm where both the α- and β-acids have low absorptivity, but a degradation product has increased absorptivity.

Ideally standards of pure α-acids, β-acids, and the degradation product would be helpful to identify the absorbance maxima and confirm the proper choice of wavelengths; however, these materials are not readily available. Reference spectra can be found in the literature and used for this purpose if needed.[1]

Analysis

Because there are two major components in the hop extract, the mixture of α- and β-acids appears to be an ideal system for a classic two-component analysis as is covered in most quantitative analysis texts. All that is needed is molar absorptivity coefficients at two different wavelengths for each component, so a system of two equations can be solved for two unknowns. This natural product extract is, however, more complex. For starters neither the α-acids nor the β-acids are a single compound. Instead they are each a family of related compounds with similar absorption characteristics. Additionally there is a third component that appears over time as the α- and β-acids are degraded. This third component has not been purified and is thought to be some other breakdown component of the hop.[1,7] It absorbs most strongly at 275 nm but has significant absorptions at 325 and 355 nm where it augments the absorption of α- and β-acids and interferes with a standard two-component analysis.

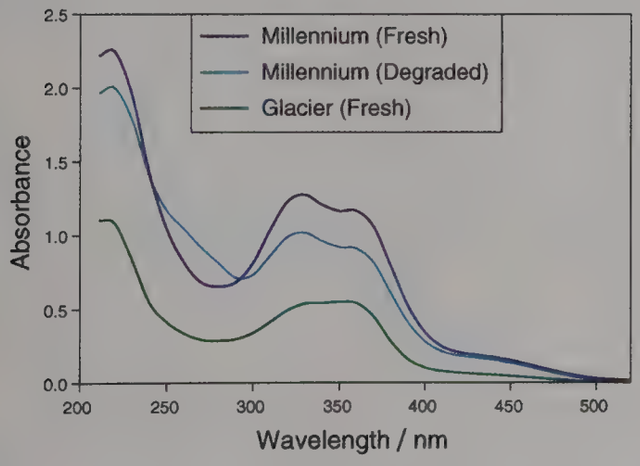

Figure 2. The UV–visible spectrum of three hop samples: Glacier hops (low in humulones), Millenium hops (high in humulones), and the same Millenium hop sample that has been degraded by storing at room temperature for one week. Key wavelengths used in this analysis are 275, 325, and 355 nm.

To a first approximation this very complex system can be studied as a three-component system if the focus is placed on the major component families, rather than on trying to analyze each individual chemical. Instead of using a molar absorptivity (units of L/(mol cm)), which relates the absorbance measurement to the molar concentration of a single compound at a given path length, this system is more easily examined using specific absorptivity (units of L/(g cm)), which, in this case, relates the absorbance measurement to that of a mixture of compounds with a total concentration of 1 g/L at a given path length.

Alderton et al.[1] found that the α-acids have specific absorptivities of 31.8, 38.1, and 9.0 L/(g cm) at 355, 325, and 275 nm, respectively. The β-acids have absorptivities of 46.0, 33.1, and 3.7 L/(g cm) at the same wavelengths, whereas the third degradation component has absorptivities of 1.0, 1.5, and 3.1 L/(g cm) at these wavelengths. Because absorbance of the solution can be found by summing the absorptivities of the individual components, and assuming a 1 cm path length so the path length term can be removed to simplify the equation, the following three equations with three unknowns can be used to describe this system

$$A_{355} = 31.8C_\alpha + 46.0C_\beta + 1.0C_{comp3} \quad (1)$$

$$A_{325} = 38.1C_\alpha + 33.1C_\beta + 1.5C_{comp3} \quad (2)$$

$$A_{275} = 9.0C_\alpha + 3.7C_\beta + 3.1C_{comp3} \quad (3)$$

where A_{355}, A_{325}, and A_{275} are the absorbancies at the three analysis wavlengths and C_α, C_β, and C_{comp3} are the concentrations (in g/L) of the α-acids, β-acids, and the third component, respectively.

Although generating the equations is easy, solving them, for many undergraduates, is not. If there were simply two equations with two unknowns, either substitution or determinants could be used to solve the system. With three or more equations the situation becomes more complicated.

One method to solve the system of three equations and three unknowns is to use programs on graphing calculators such as the SIMULT function on TI-85 calculators or a the Simultaneous Equation Solver Application that can be downloaded for free for TI-83 or TI-84's from the Texas Instruments Web site.[8] Although this method finds an answer, it is not educationally satisfying because the student uses the calculator as a "black box" to find the solution without understanding the method used to obtain the answer. A better procedure is to use this system as an introduction to the method of Gaussian elimination. Once the students understand the Gaussian elimination method, they know how their calculators actually work, and they have a tool that they can use to solve virtually any system containing an equal

Table 1. Analysis of Spectra Shown in Figure 2

	absorbance data			concn/ (g L^{-1})		
hop sample	355 nm	325 nm	275 nm	α-acids	β-acids	third component
Glacier (fresh)	0.55	0.51	0.29	0.0037	0.0078	0.073
Millennium (fresh)	1.17	1.27	0.66	0.0208	0.0079	0.143
Millennium (degraded)	0.91	1.01	0.87	0.011	0.0069	0.24

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number of unknowns and equations. An introduction to Gaussian elimination is found in the Supporting Information. Because many of the manipulations used in Gaussian elimination are more easily handled using matrix manipulations, this system can be further extended to introduce the advanced students to the basics of linear algebra. Table 1 gives the experimental absorptivities of the samples shown in Figure 1 at the analysis wavelengths of 275, 325, and 355 nm, and the amounts of α- and β-acids (humulones and lupulones) and third component found using the above three-component analysis.

Yet a third solution to this educational problem, and one that could be used in a nonmajors chemistry lab with no algebra, is to use the following equations:

$$C_\alpha = -0.05156A_{355} + 0.07379A_{325} - 0.01907A_{275} \quad (4)$$

$$C_\beta = 0.0555A_{355} - 0.04759A_{325} + 0.00510A_{275} \quad (5)$$

$$C_{comp3} = 0.08336A_{355} - 0.1574A_{325} + 0.3719A_{275} \quad (6)$$

Equations 4 and 5 are similar to those given in Alderton et al.,[1] but the coefficients are 1000 times smaller because Alderton's equations yield concentrations in mg/L. Even this simple, nonmathematical approach has its use in the more advanced lab. First, it can be used to check the answer obtained using more advanced algebra. Second, as shown in the Supporting Information, the above equations can be obtained by taking the inverse of the matrix formed from eqs 1−3. This is another calculation that the students can do easily on their calculators or in Excel, but requires a background in linear algebra to do rigorously. If the instructor wishes to take the more rigorous approach, the inversion matrix can be found by extending the Gaussian elimination to a complete Gauss−Jordan diagonalization followed by normalizing the diagonal matrix.

In the end, the brewer is not interested in knowing the concentration of α- and β-acids in an extract, but rather, wants to know the percentge of α- and β-acids in the actual dried hop. Thus, the final task for the student is to work back through the dilutions to find this number. The way the procedure is designed, if exactly 2.5 g of hops is extracted with 50 mL of methanol, the number for concentration of α- or β-acid found in the final extract expressed in mg/L is exactly the same as the percentage of α- or β-acid in the hop material. It should be noted that results for the third component occasionally yield negative values, indicating that this component is not yet completely understood.

For additional usefulness, this lab can be performed in conjunction with a laboratory that uses HPLC to analyze hops for α- and β-acids[9] so the same material can be analyzed by two different instrumental methods and the strengths and weakness of the two methods compared.

■ CONCLUSIONS

Laboratories dealing with hops or brewing spark instant interest in most students. This lab is particularly useful because it can be performed with different levels of sophistication from a nonmajors class up to an instrumental class with an involved discussion of the mathematical methods used to solve a system of multiple equations and unknowns. In our locale where we have both microbreweries and hop growers, the techniques introduced in this lab will be used in student research projects for the next several years.

■ ASSOCIATED CONTENT

Supporting Information

A lab procedure with instructor notes; an extensive math review. This material is available via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

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Charge Density Quantification of Polyelectrolyte Polysaccharides by Conductometric Titration: An Analytical Chemistry Experiment

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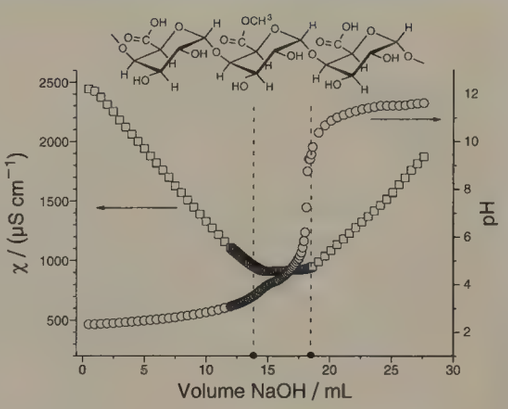
Supporting Information

ABSTRACT: An easy analytical method for determination of the charge density of polyelectrolytes, including polysaccharides and other biopolymers, is presented. The basic principles of conductometric titration, which is used in the pulp and paper industry as well as in colloid and interface science, were adapted to quantify the charge densities of a negatively charged polysaccharide (pectin) and a positively charged biopolymer (chitosan), two biomacromolecules commonly used in food and biomaterials applications. This novel conductometric titration method can be easily applied in most analytical chemistry teaching laboratories, due to its ease-of-use, safety, and educational benefits. This analytical technique can also be used in a wide-range of laboratory activities and has extensive research applications in areas of chemistry involving charged biopolymers, such as food science, materials science, and physical chemistry.



KEYWORDS: Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Carbohydrates, Conductivity, Food Science, Materials Science, Titration/Volumetric Analysis

Titration is a commonly used analytical technique in many research and classroom laboratory activities and is defined as the addition of a solution with known concentration (the titrant) to a second solution with an unknown concentration (the analyte), with the goal of determining the concentration of the latter. Titration is complete when a specific end point is reached. There are several methods for end point determination: pH indicators (e.g., phenolphthalein), redox indicators, pH meters, conductometers, potentiometers, ζ-potential, isothermal calorimeters, spectrophotometers, and amperometric instruments.

This article focuses on conductometric titration, a titration technique based on measuring conductance changes during stepwise addition of a titrant to an analyte. The conductivity of a solution depends on several factors, including solute concentration, the degree of solute dissociation, the valence of the ion(s) present in the solution, temperature, and the mobility of the ions in the solution. Conductometric titration is a versatile technique, with a wide range of applications. It is a well-established analytical method for simple acid−base systems[1,2] and has recently been applied to analyze biological molecules for various purposes.[3−5] In addition, conductometric measurements are routinely conducted in the pulp and paper industry to assess the mechanical performance of paper by absorption of additives onto the fiber surface, the deposition of colloidal materials, such as small cellulose fragments and filler particles; or when stoichiometric neutralization of anionic trash is required.[6,7] The potential usefulness of conductometric titration as a routine laboratory technique has been proposed in textbooks over 20 years ago.[8] Moreover, the basic principles behind related conductometric titration apparatus have been described in this *Journal*.[9−14]

In light of the rapidly increasing interest in the use of biomacromolecules for a broad spectrum of practical applications, in the present work an easy-to-use conductometric titration method to quantify the charge density of biomacromolecule polyelectrolytes is described. The charge density, defined as the amount of electric charge per mass unit, provides a quantitative measure of the charged groups along the molecular backbone of a biomacromolecule. These groups may be either positively charged or negatively charged. There are several methods for the determination of the charge density, among which electrophoretic and light scattering techniques, colloidal titation, and pH titration are the most widely exploited. In this article, the advantages of conductometric titration for determination of the charge density of polyelectrolyte biopolymers are illustrated. The educational goal of this work is to introduce students to the fascinating world of biomacromolecules and to teach students how to manipulate and investigate their versatile nature using a simple analytical technique. The student experiments have been successfully used for teaching purposes, in particular within lab activities of undergraduate students of the food science program. The entire activity includes three parts: (i) setup of the experiment (~30 min), (ii) carrying out the conductometric titration experiments (~150 min), and (iii) data analysis (~30 min).

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Figure 1. Equipment required for conductometric titration.

■ EXPERIMENTAL PROCEDURE

Materials

All raw materials can be readily purchased from chemicals suppliers: 0.1 N hydrochloric acid (HCl), 0.1 N sodium hydroxide (NaOH), and low-methoxyl pectin from citrus with a degree of esterification (DE) = 28.5%, were purchased from Sigma-Aldrich (Milan, Italy). Medium molecular weight shellfish chitosan was provided by Giusto Faravelli Spa (Milan, Italy). The degree of acetylation, DA (%), of the chitosan was approximately 16.13%.

Sample Preparation

Pectin, an anionic polyelectrolyte, and chitosan, a cationic polyelectrolyte, must be dispersed in water before use. A highly dilute dispersion (e.g., 0.1 wt %) can be prepared in approximately 30 min (see Supporting Information).

Conductometric Titration Instrument Setup

To simultaneously monitor the pH and conductivity of the prepared dispersion, a pH meter and conductometer must be carefully fixed close to the beaker containing the water dispersion (the analyte). A magnetic stirrer is used to continuously mix the water dispersion throughout the experiment. Because both pH and conductivity are strongly influenced by temperature, a temperature-controlling device is used to ensure that this parameter is constant during analysis. In addition, because the titrant should be dispensed precisely, the use of an automatic microburet is recommended. However, for the purposes of the student laboratory exercise, a manual buret is sufficient. A typical instrument setup is illustrated in Figure 1. Setup will normally require approximately 30 min.

■ ANALYSES

Conductometric titration of both biopolymers requires approximately 2.5 h.

Conductometric Titration of Pectin

A pectin aqueous dispersion (0.1 wt %) was first treated with an excess (15 mL) of 0.1 N hydrochloric acid (HCl), to completely neutralize the negative charge distributed along the pectin backbone from the unprotonated carboxylic groups. Conductometric titration was performed by adding 0.1 N sodium hydroxide (NaOH) under gentle stirring (100 rpm). Ionic conductivity was evaluated after sequential injections of NaOH in three stages: (i) initially, 0.5 mL drops were dispensed at a flow rate of 0.40 μL s^{-1}; (ii) as the conductance decreased (approaching the first equivalence point), the dispensed volume was reduced to 0.1 mL at a flow rate of 0.15 μL s^{-1}; and (iii) beyond the "constant-conductivity" region, 0.5 mL drops were dispensed at a flow rate of 0.40 μL s^{-1}. The titrant was added approximately every 60 s, to allow sufficient time for equilibrium to be reached between readings. pH was continuously measured simultaneously.

Conductometric Titration of Chitosan

Conductometric titration of chitosan aqueous dispersion (0.1 wt %) was performed without any prior neutralization step. Hydrochloric acid (0.1 N HCl) was added approximately every 2 min in two stages: (i) initially, 0.1 mL drops were dispensed at a flow rate of 0.15 μL s^{-1} and (ii) as the conductance increased (after the breakpoint), the dispensed volume was increased to 0.5 mL, with a flow rate of 0.40 μL s^{-1}. Ionic conductivity was evaluated after each addition of titrant, and pH was continuously measured.

Charge Density Determination

The charge density (as equivalent charge) of the polyelectrolytes can be determined by plotting the measured ionic conductivity versus total titrant. From the intersection points of the linear segments of the ionic conductivity plot before and after the equivalent point (or breakpoint), it is possible to graphically determine the volume (mL) of titrant required to fully deprotonate all carboxylic groups on pectin or fully protonate all amino groups on chitosan. By multiplying this value by its concentration (normality), and referring to the initial polyelectrolyte mass, the charge density of the polymer (mmol g^{-1}) can be calculated. A detailed example is reported in the Supporting Information. This step normally requires 30 min.

■ HAZARDS

The conductometer, pH meter, and data logger are safe to use; however, high voltage power supplies must be used with caution. Concentrated sodium hydroxide solution is caustic; although high temperatures may result upon mixing sodium hydroxide with water, this was not observed during the stepwise sodium hydroxide titration described above. Both hydrochloric acid and sodium hydroxide solutions should only be handled (filling, closing, and shaking) while wearing a protective lab coat, gloves, and safety glasses. The pectin and chitosan used in these experiments are safe and are used as food ingredients. However, very low mesh powders may be irritating to the respiratory tract. Therefore, a protective mask is recommended during handling.

■ RESULTS AND DISCUSSION

Pectin

Typical plots of ionic conductivity (χ) and pH versus the volume of titrant for dilute aqueous pectin dispersions are shown in Figure 2. Both curves clearly display three distinct zones, corresponding to three distinguishable physicochemical phenomena. In zone 1, the first descending part of the conductometric curve is due to neutralization of dissociated hydrogen ions from the previously added HCl (Figure 3A), as shown by

$$NaOH + H^+ + Cl^- \leftrightharpoons H_2O + Na^+ + Cl^- \quad (1)$$

Because the equivalent ionic conductance of H^+ (350 S cm^2 mol^{-1}) is approximately 7 times greater than the mobility of Na^+

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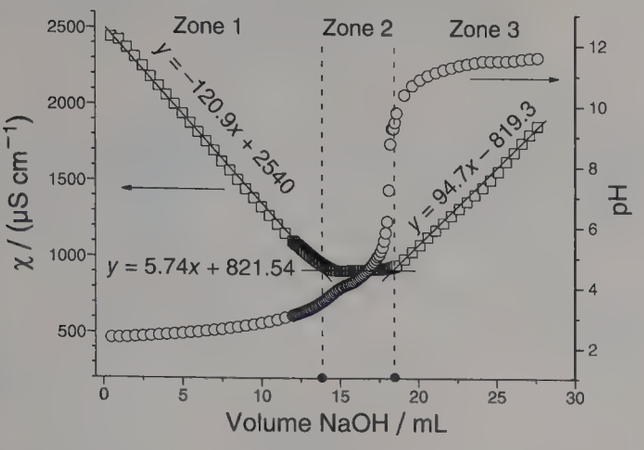

Figure 2. Mean conductometric and potentiometric titration curves for pectin (DE = 28.5%).

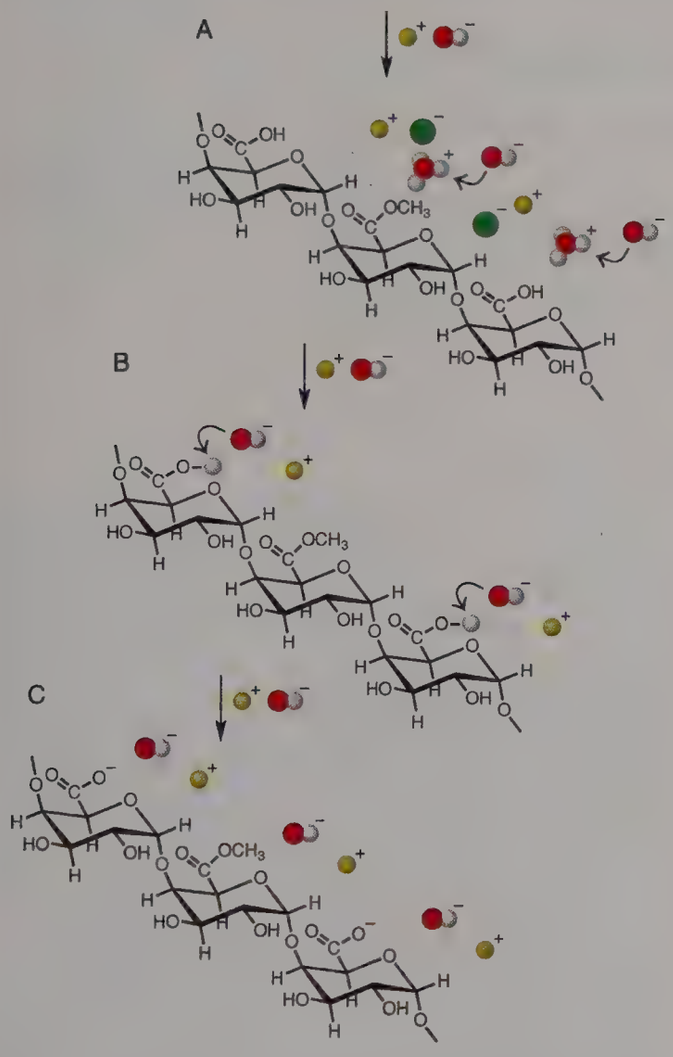

Figure 3. Schematic representation of chemical changes induced by NaOH titration of the pectin backbone: (A) neutralization of hydrogen ions (excess HCl) by NaOH; (B) early dissociation of carboxylic groups mediated by hydroxide ions; and (C) from pectic acid to pectate, dissociation of all carboxylic groups. Na = yellow, O = red, H = white, and Cl = green.

(50.9 S cm^2 mol^{-1}),[15,16] the net effect is a decrease in conductivity. At the same time, the pH increases moderately as the concentration of hydrogen ions decreases.

As the first equivalence point is approached, neutralization of the excess H^+ is complete and carboxylic acid groups began to

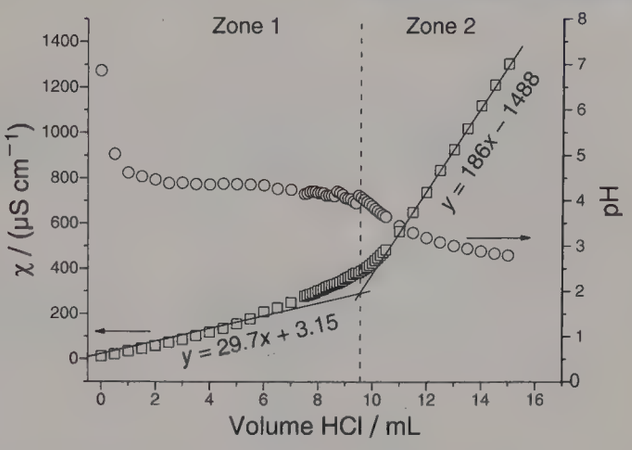

Figure 4. Mean conductometric and potentiometric titration curves for chitosan (DD = 83.87%).

dissociate (zone 2). In this zone, no changes in conductance values occur because of the neutralization of dissociated hydrogen ions from the pectic acid backbone by hydroxide ions (OH^-), which arise from the addition of titrant (Figure 3B) according to

$$-COOH + Na^+ + OH^- \leftrightarrows -COO^- + Na^+ + H_2O \quad (2)$$

Simultaneously, the pH of the pectic acid dispersion progressively increases as deprotonation of the carboxylic groups proceeds, due to increasing quantities of free OH^- ions in the dispersion.

Zone 3 begins beyond the second equivalence point. Because carboxylic groups have been titrated, the conductance increases in proportion to the OH^- excess in the dispersion, arising from dissociation of the NaOH dispensed (the hydroxyl equivalent ionic conductivity is ~192 S cm^2 mol^{-1}) (Figure 3C). At this point, addition of even small volumes of titrant will yield a dramatic increase in pH, until NaOH dissociation becomes progressively hindered by higher concentrations.

According to the graphical method described in the previous section, the final anionic charge density of pectin is 2.471 ± 0.123 mmol g^{-1} or approximately 0.492 mmol/0.2 g of pectin.

Chitosan

The degree of acetylation, DA (%), of the chitosan used in this work was approximately 16.13%, corresponding to a degree of deacetylation (DD) of ~83.87%. A typical evolution of ionic conductivity (χ) and pH versus volume of titrant for dilute aqueous chitosan dispersions is shown in Figure 4. Both curves clearly display two distinct zones. Initially, the pH of the chitosan dispersion is above the pK_a of chitosan (~6.5), with the absence of any predominant charge along the polysaccharide molecule; thus, all amino groups ($-NH_2$) are in their unprotonated form. The absence of positive charge greatly influences the physical properties of chitosan dispersions by affecting its solubility in water. In support of this, chitosan dispersions prepared in this work initially appeared very cloudy (i.e., high turbidity) due to the presence of insoluble particles of solute dispersed in the solvent. The lack of solubility of native chitosan in water has been ascribed to its inherent physical structure. Specifically, its β-1,4-configuration results in a rigid and unbranched structure, whereas the abundance of hydroxyl groups (one primary hydroxyl and one secondary hydroxyl) and a highly reactive amino group explain the tendency for intra- and intermolecular hydrogen

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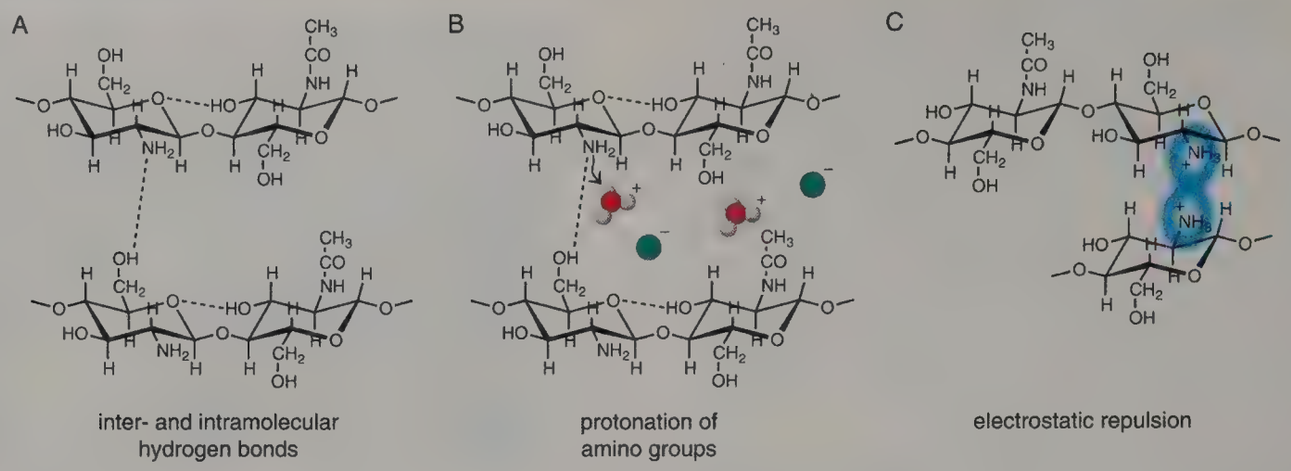

Figure 5. Schematic representation of chemical changes induced by HCl titration of the chitosan backbone: (A) undissolved chitosan with unprotonated amino groups (dashed lines indicate the intra- and intermolecular hydrogen bonds); (B) partial protonation of amino groups is promoted by the addition of HCl (figure shows the hydronium ion H_3O^+ and the chloride ion Cl^-); (C) full protonation of amino groups leads to complete solubility of chitosan because of increased polarity and electrostatic repulsion.

bond formation (Figure 5A). Increasing volumes of titrant (HCl) gradually lead to an increase in the solubility of chitosan, with a simultaneous increase in the transparency of the chitosan dispersion. This is due to protonation of amino groups ($-NH_3^+$), which promotes unfolding of the chitosan molecules by electrostatic repulsion (Figure 5B). Simultaneously (with the exception of an initial decrease in pH, presumably because of system stabilization), the pH values of the chitosan dispersion remain steady up to the equivalence point, due to continuous protonation of $-NH_2$ groups, while conductivity values increase slightly, because of the release of free chloride anions (Cl^-). The end of the first zone is assumed to correspond to the total protonation of amino groups, and maximum transparency of the chitosan dispersion is attained at this point (no chitosan particles can be seen by visual inspection). Further addition of hydrochloric acid prompts a steep increase in conductivity, due to the larger conductivity of hydrogen cations (350 S cm^2 mol^{-1}) compared to chloride anions ions (75.5 S cm^2 mol^{-1}) (Figure 5C).

This is also consistent with slope values from the two distinct linear segments of the conductivity curve. The slope of the first linear segment of the conductivity curve is approximately 6-fold greater than the slope of the second linear segment (~30 vs ~185 μS mL cm^{-1}), in agreement with the smaller equivalent conductivity of Cl^- ions (75.5 S cm^2 mol^{-1}) compared to the H^+ ions (350 S cm^2 mol^{-1}). Accordingly, pH values start to decrease from this point on, and throughout the second zone, due to increasing quantities of free H^+ ions in the medium. On the basis of these observations, the equivalence point is located at the intersection of the two linear segments of the curve, from which the corresponding volume of titrant used can be extrapolated. The calculated anionic charge of chitosan was 4.660 ± 0.056 mmol g^{-1}, or 0.932 mmol/0.2 g of sample.

■ CONCLUSIONS

The results demonstrate that this conductometric technique is a valid method for quantifying the charge density of dilute solutions of polyelectrolyte polymers, such as polysaccharides. This tool could be especially relevant when assembly of biopolymers governed by electrostatic forces is required. In addition, because this technique is relatively rapid, safe, and easy-to-use, it can be successfully adapted for teaching laboratories for undergraduate student courses.

■ ASSOCIATED CONTENT

Supporting Information

Teacher and student guide to conductometric titrations. Full details of the operative conditions. This material is available via the Internet at http://pubs.acs.org.

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Microfluidic Gel Electrophoresis in the Undergraduate Laboratory Applied to Food Analysis

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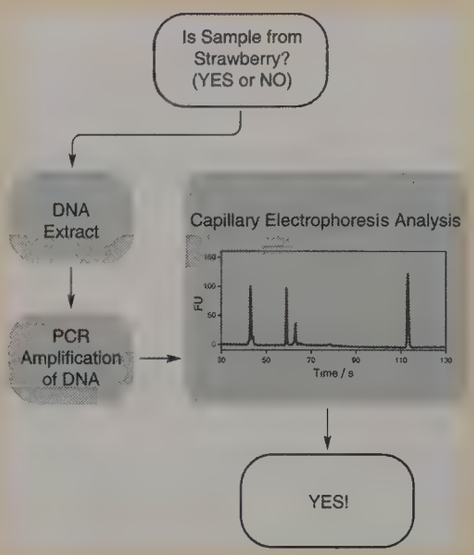
Supporting Information

ABSTRACT: A microfluidics-based laboratory experiment for the analysis of DNA fragments in an analytical undergraduate course is presented. The experiment is set within the context of food species identification via amplified DNA fragments. The students are provided with berry samples from which they extract DNA and perform polymerase chain reaction (PCR) with strawberry-specific primers. The resulting PCR products are analyzed using the Agilent Bioanalyzer. Using the raw data, the students are tasked to identify the strawberry sample. This course serves as a practical introduction into microfluidic-based capillary gel electrophoresis as well as a primer for biomolecular DNA analysis.

KEYWORDS: Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Bioanalytical Chemistry, Electrophoresis, Food Science, Microscale Lab, Nucleic Acids/DNA/RNA, Separation Science



Since its early analytical applications in the 1990s, microfluidic systems or labs-on-a-chip are becoming more standard in analytical laboratories.[1−3] Components necessary to perform microfluidic analyses are now commercially available, such as programmable multichannel power supplies and detection systems, as well as the microfluidic manifolds. Even complete commercial microfluidic separation systems can now be purchased. Because of their numerous advantages, including low cost, high throughput, and high speed of analysis, microfluidic systems are expected to become an important tool in modern analytical procedures.[4] Training future chemists on this novel technology as part of the undergraduate curriculum will thus endow graduate students with scientific knowledge and laboratory skills needed for the 21th century.

An undergraduate microfluidic experiment is described, which was developed for an instrumental analysis laboratory module and employees a complete commercially available platform. An important aspect of instrumental laboratory training for undergraduate students is to provide a practical context for the used techniques. Thus, a course module is presented capable of teaching basic microfluidic-based capillary gel electrophoresis (CGE) with an application related to food analysis. The chosen application further teaches basic molecular biological methods for food testing with a special focus on the use of DNA size analysis. These experiments can be easily integrated into courses teaching analytical techniques, but can also be part of molecular biology courses. Moreover, this laboratory module has a high degree of interdisciplinarity due to the combination of biochemical techniques with novel instruments.

The laboratory module presented is taught in the instrumental analysis laboratory course in which spectroscopic and separation techniques are covered. Students rotate three assignments each half of the course. The first half of the course consists of a Fourier transform infrared spectroscopy, a UV−vis, and an HPLC module. These three modules serve as the basis for introducing students into instrumental techniques, on which they should have basic theoretical knowledge at this point, but also to introduce them to laboratory practices, prelab reports, and requirements, as well as the final laboratory reports. The second half of the class provides more insight in analytical instrumentation and more challenging analytical concepts consisting of a cyclic voltammetry, a GC−MS, and the miniaturized capillary electrophoresis experiment as presented in this manuscript. The complete course is taught in one semester, laboratory times are 4 h per session, and each experiment is assigned for two sessions.

EXPERIMENTAL OVERVIEW

In an age where processed foods are becoming dominant components of regular diets[5] and the food market has become globalized, the control of authenticity and safety of food is gaining in importance. One of the main tools for food testing is the detection of specific DNA sequences that are routinely used

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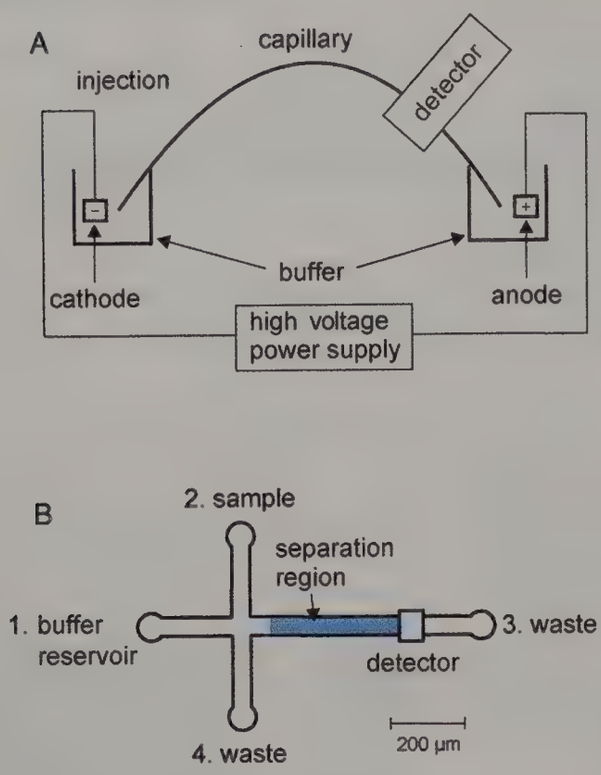

Figure 1. Schematics comparing (A) capillary electrophoresis and (B) microchip capillary electrophoresis. Required components of a capillary electrophoresis system are an injector, a circular microcapillary, and a detector. High voltage is applied via electrodes immersed in the sample and waste reservoir into which the capillary is dipped. Injections can be accomplished via pressure, suction, or electrokinetically via application of a voltage pulse. Commercial capillary electrophoresis systems are usually benchtop systems in the size of a dishwasher. In microchip capillary electrophoresis, the injection of nL–pL sample plugs is accomplished via a network of channels intersecting at the injection position. The most common injection schemes are performed electrokinetically. Microchannels (not necessary circular) integrated on a planar microchip serve as the separation capillary. The detection principles are similar to capillary electrophoresis, however, are adapted to suitably perform in the given channel geometries. Microchips are only a few cm^2 in size and used in commercial benchtop systems the size of a desktop computer.

to determine, for example, food species, genetically modified crops, and bacterial contamination.[6−11]

Students are challenged to confirm the presence or absence of strawberries (*Fragaria* sp.) in a food sample. The identification of strawberries is based on the detection of specific simple sequence repeats (SSRs), also termed microsatellites. SSRs are arrays of short repeating genomic sequences with variable lengths, which are ubiquitous in eukaryotic genomes. These genomic regions are hypervariable and often exhibit high allelic variations even among closely related strains. This can be used for a variety of population-based genetic analyses, including the assessment of relationships or the identification of a specific individual. The detection and analysis of SSRs is facilitated by amplifying the respective SSR or sets of SSRs by polymerase chain reactions (PCRs) with specific primers, and subsequent product analysis by slab gel or CGE. The primers used in this study amplify SSRs in strawberries with the base motif $(GA)_{16}$ and $(GA)_{20}$ (i.e., consisting of repeats of the indicated sequence), respectively. They were successfully detected in woodland strawberry (*Fragaria vesca*), beach strawberry (*Fragaria chiloensis*), the Virginia strawberry (*Fragaria virginiana*), and the garden strawberry (*Fragaria* × *ananassa*), a hybrid between

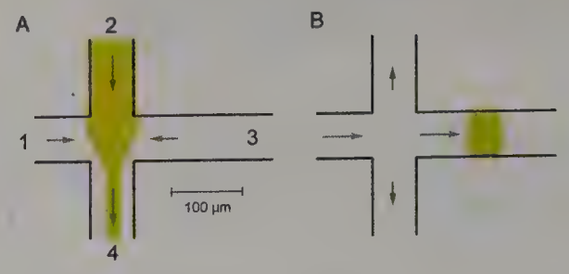

Figure 2. Demonstration of the pinched, electrokinetic injection for a channel system consisting of four channels. (A) During the loading step, the sample flows from the sample channel 2 to the sample waste channel 4, with additional "pinching flow" from channels 1 and 3 to confine the liquid at the intersection. (B) In the injection step, the flow is directed from channel 1 across channel 3 leading to the injection of a plug approximately the dimensions of the intersection toward the analysis part of the microfluidic device. The injected volume is typically in the pL to nL range. Microchannel widths are typically 50−100 μm.

F. virginiana and *F. chiloensis*. Moreover, the primers were found to be able to detect polymorphisms (and thus strain differences) in *F.* × *ananassa* and *F. virgiana*.[12,13]

The most common method in laboratory courses is to analyze the DNA with agarose or polyacrylamide gel electrophoresis. However, especially in commercial settings, CGE is used as a faster, automated, high-resolution alternative to slab gels. Introducing students to this technique is therefore of high relevance. A disadvantage of capillary electrophoresis experiments is that they are often challenging to implement into short units, especially in basic courses with a high number of participants. Quite often this is resolved by demonstrations conducted by the instructors. However, hands-on experience generally results in a deeper understanding and interest in the technique being taught. Chip-based capillary gel electrophoresis systems such as the Agilent Bioanalyzer or BioRad Experion System are an attractive alternative to traditional CGE systems in student's courses. Such instrumentation can run a large number of samples in a comparatively short time and does not require expert knowledge to operate.

CGE follows the same principles as capillary zone electrophoresis, which have been described in the context of a student's course elsewhere.[14,15] However, capillary zone electrophoresis generally does not provide sufficient resolution to differentiate between DNA of different lengths. This is because the electrophoretic mobility of DNA only changes within a narrow size range, outside of which it becomes size-independent.[16,17] This limitation is generally overcome by introducing a molecular sieving effect by filling the capillary or DNA chip with a suitable matrix that can be adjusted to the desired dynamic range.

Microchip capillary electrophoresis (MCE) differs from capillary electrophoresis (CE) in some major parts (Figure 1). In MCE, the separation capillary is replaced by a channel on a planar material. The cross-sectional shape of this channel is determined by the microchip material as well as the microfabrication method. Most often, rectangular cross sections or semicircular channels are found. Detectors in MCE can be very similar to CE and are usually situated at the end of a separation channel. They can be realized as, for example, absorbance, fluorescence, or electrochemical detectors. Because of the relatively basic optical instrumentation required for moderate to high sensitivity, fluorescence detectors are often employed, as is the case in this experiment with the Bioanalyzer instrument.

One important difference between traditional CGE and chip-based electrophoresis systems is the means of sample injection.

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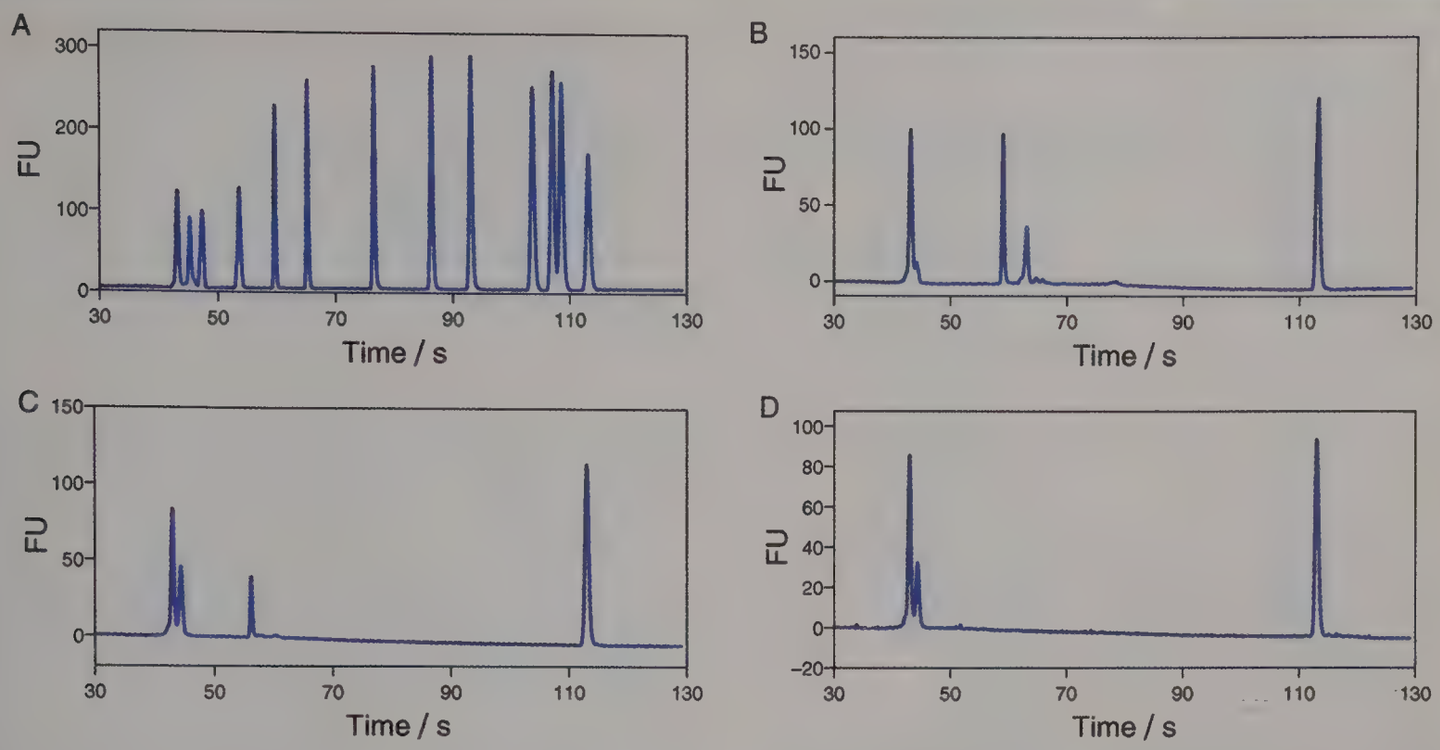

Figure 3. Electropherograms from a typical Agilent Bioanalyzer run: (A) size standard, (B) PCR product with strawberries as template and *Fvi*20 as primers, (C) PCR product with strawberries as template and *Fvi*11 as primers, and (D) PCR result with raspberry as template and *Fvi*20 as primers. The first and last peak in each electropherogram are internal size standards. The signal intensity is given in arbitrary fluorescence units (FU).

In CE, samples are directly delivered via pressure-driven or electrokinetic injection into the separation capillary. In contrast, samples are loaded via channel intersections in MCE.[18] To accomplish electrokinetic injections on a microchip, electrodes are dipped into the reservoirs, thus, allowing for the application of high voltage to the individual channel. Bulk flow based on electroosmosis can thus be accomplished. The frequently used pinched-injection process on a planar microchip with four channels is described in Figure 2. The analyte is driven electrokinetically evoking bulk flow into the intersection from the sample well 2 on the chip via application of an electric field from well 2 to well 4. To confine the injection volume in this intersection, an additional electric potential is applied at wells 1 and 3. This ensures bulk flow from 1 and 3 into the intersection, thus, confining or pinching the analyte in a typical V-shape at the injection position. By application of electrical potential between 1 and 3, the volume in the intersection is injected and separation starts. With this method, typically picoliter to nanoliter analyte plugs are injected.

This instrumentation demonstrated in this laboratory experiment gives students hands-on experience on DNA separation and allows the instructors to explain and demonstrate the principles of CE along with basic principles of separations in microfluidic systems.

■ EXPERIMENTAL PROCEDURE

The described experiment is designed such that it can be performed in two periods each consisting of roughly 3 h of experimentation and 1 h of instruction and explanation. Although it is possible to successfully conduct the same experiment with processed food, it is advisable to use fresh or frozen berries to ensure a successful outcome. Either fresh or frozen strawberries from commercial sources are used, which are likely to be *F.* × *ananassa* variants. As different strawberry strains may yield slightly different PCR fragment sizes due to variations in their SSRs, it is recommended to determine the expected fragment sizes prior to the start of the course. To keep the course challenging for the students, a sample with blended raspberry (*Rubus* sp.) is also prepared, which is visually similar to strawberries but which does not yield a diagnostic PCR product.

At the beginning of the lab session, students are assigned to two-person teams. Each team produces a lysate from the assigned samples and is then tasked to correctly identify the strawberry sample. For the cell lysis and DNA extraction, commercial kits (such as, e.g., FastDNA SPIN Kit for plant and animal tissues, MP Biomedicals) can be used, which generally result in satisfying results in the hand of novices and avoids the use of organic solvents.

Following DNA extraction, the yield and purity is determined by UV spectroscopy. The absorbance at 260 nm is used to determine the DNA concentration whereas a measurement at 280 nm determines impurities. The A_{260}/A_{280} ratio should be 1.7−2.0 for the DNA extraction. PCR is performed using the primers *Fvi*11 and *Fvi*20 (see ref 12 and Table 1 in Supporting Information) that target a SSR with an expected size of 114−292 base pairs (bp). The whole process including the preparation of the PCR requires around 2 h. During the PCR run, additional instructions can be given as to the principles of the subsequent DNA analysis methods. Following completion of the PCR, the samples are removed by the instructors and frozen until the next laboratory period.

In the second laboratory session, students analyze the PCR products using the Agilent Bioanalyzer system. This system is available in the student labs, but alternatively the same analysis can be performed on other commercially available systems such as the Experion System from BioRad. As relatively low PCR product sizes are expected (ca. 100−300 base pairs), the DNA 1000 kit reagents should be used. The chip is prepared and loaded according to the manufacturer's instructions. A total of 12 samples can be analyzed in a single run, which requires around 30 min.

Table 1. Size Markers and PCR Fragments and the Related Theoretical Plate Number

DNA Size/bp	Theoretical Plate Number
140 (*Fvi*20)	134144
163 (*Fvi*20)	112456
126 (*Fvi*11)	117283
25	45074
50	170381
100	52910
150	161184
200	147644
300	129855
400	136239
500	124784
700	120530
850	169027
1000	115090

The Agilent Bioanalyzer provides finalized reports with calculated fragment sizes. However, for educational purposes, it is advisable to supply the students with the trace data only. On the basis of the gel images and the electropherograms, the students are tasked to determine fragment sizes by preparing calibration curves and finally decide which of the tested samples contained strawberries. Furthermore, the electropherograms are also used to calculate and explain relevant parameters in CE such as the separation efficiency (theoretical plate number) and reproducibility of the results.

■ HAZARDS

Microchip CE requires high voltages. Safety precautions are in place with the commercial instrument used. However, students should be advised of the involved risks of accidental shocks. Hazards in this experiment are mainly the components of the DNA isolation and the DNA chip kits. Especially the gel-dye mix should be treated as potentially mutagenic. As precaution, lab coats, gloves, and eye protections should be worn throughout the experiments. Contaminated materials and chips should be disposed appropriately as hazardous chemicals.

■ RESULTS AND DISCUSSION

A typical result in an undergraduate course is presented in Figure 3. Shown are (A) the electropherograms for the size standards (see Table 1 for the fragments included in the size standard), (B) the PCR products with strawberries as template and *Fvi*20 primer, (C) the PCR products with strawberries as template and *Fvi*11 primer, and (D) the PCR products with raspberry as template and *Fvi*20 primer. The first and last peaks in each run are internal standards. Also note that in the raspberry sample, but to a lesser extent also in the strawberry samples, a weak signal at around 43 s can be observed (corresponding to ~40 bp), which is likely caused by primer dimers. The students prepared a calibration curve with the electropherograms of the size standards as shown in Figure 4 from which the sizes in bp of the obtained PCR products were calculated. This regression line for the calibration was obtained in the linear range from 100−1000 bp length of the size standards resulting in a coefficient of determination R^2 of 0.9889. With *Fvi*20 primers,

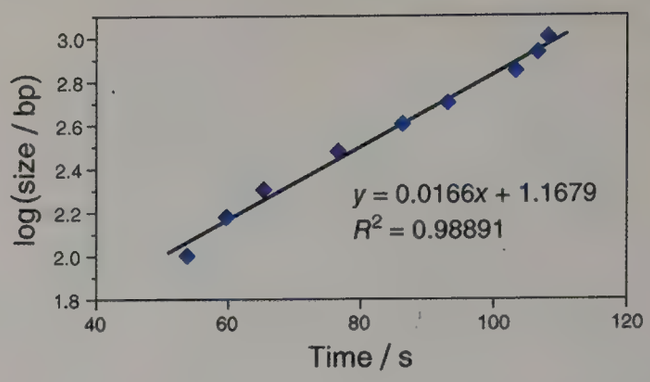

Figure 4. Calibration curve for the determination of unknown raspberry and strawberry fragments. The resulting regression line is calculated in a size range from 100 to 1000 bp fragment size and R^2 for a linear fit on a semi-logarithmic plot is given.

two products at 58.9 and 62.9 s corresponding to sizes of 140 and 163 bp, respectively, were detected as obtained from the calibration curve. The *Fvi*11 primers yielded a single product only of 126 bp. The manual calibration procedure and fragment size assignment only differs by a maximum of 4 bp compared to the Bioanalyzer's automated software. According to the manufacturer's specifications, the typical size resolution in this ranges is about ±5%. On average, within a typical course the size ranges fall within 174−189 bp with an average of 182.25 and standard deviation of 6.36. Furthermore, with neither primer pair a product was observed with raspberry DNA as template, see Figure 1D exemplarily for the *Fvi*20 primers. Earlier reports indicated that with these primers a few *Rubus* strains (e.g., raspberries and blackberries) may yield a single product.[13] However, even then these samples can be easily distinguished from the multiple products generated with strawberry DNA as template. The data thus indicated that commercial available *Rubus* strains are well suited for a student experiment due to the unequivocal distinction between strawberry and raspberry.

Treating the peaks as Gaussian, the theoretical plate number can be calculated as

$$N = 5.54\left(\frac{t_m}{W_{0.5}}\right)^2 \quad (1)$$

where t_m is the migration time and $W_{0.5}$ is the full width at half maximum.[19,20] The theoretical plate number for the whole range of size markers is found between 45,074 and 161,184, shown in Table 1. In addition, the collected data can be used to assess reproducibility of the DNA chips by calculating relative standard deviation between samples within a run and also between runs (if conducted).

The electrophoretic mobility, μ, of an analyte can be obtained in a CE experiment from the migration time t_m and the applied field

$$\mu = \frac{L_d}{t_m E} \quad (2)$$

where L_d is the length from the injector to the detector and E the applied electric field. This relation for μ can be simplified if the detector is situated at the end of the capillary, and thus, L_d equals the total capillary length. Then,

$$\mu = \frac{L_d^2}{t_m V} \quad (3)$$

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with V, the applied voltage. Typical results for the mobility for the shorter strawberry fragment with the *Fvi*20 primers resulted in an electrophoretic mobility of 3.4×10^{-4} $cm^2/(Vs)$. This is in reasonable agreement with literature reporting electrophoretic mobilities of DNA in that size range and in polymer matrices from 1.5 to 3.5×10^{-4} $cm^2/(Vs)$.[21] Note that a contribution of electroosmotic flow to the overall migration is neglected.

Overall, this microfluidic experiment was found to be an excellent tool to provide hands-on experience on capillary gel electrophoresis and DNA analysis, as well as molecular biological analyses relevant to food testing.

This course can be adapted to a stronger emphasis in genotyping, by using different strawberry strains. In this case, it may be necessary to employ additional primer pairs (see Table 1 in the Supporting Information) and thus additional PCRs for reliable identification. Depending on the available strawberry strains, optimization of the reactions is necessary. The main body of the experiments remains unchanged in this scenario. Very similar to the present experiment, a lab module detecting genetically modified food samples or to demonstrate forensic DNA testing using the student's own DNA can be developed with this approach. The protocols for these experiments have been published earlier[22,23] and can be easily combined with microchip analyses. Further application of this module to protein electrophoresis is also possible, employing customized microchips and kits provided by instrument suppliers.

Finally, the challenges and the performance of students in this laboratory module are noted. Students are instructed to write a scientific report that follows ACS style for journal articles and are given specific keywords to facilitate their scientific article search. Generally, students performed satisfactory with this task. Some students faced the challenge of integrating the related research articles in the introduction with proper citations. Others were challenged with segregating the "discussion" from the "conclusion". The latter was a common mistake. Writing a brief and comprehensive abstract was also problematic for some students. In spite of the challenges faced by the students to write these reports, the process introduces them to perform scientific journal search with relevant keywords and databases. The overall student performance with the laboratory report was satisfactory as expected from undergraduate students in their third or fourth year.

The course was composed of either students having strong biology background or strong analytical chemistry background. For students with biological background, understanding the tissue extraction and PCR was not a challenge as well as understanding the basics of electrophoresis of DNA in microfluidic chip. The students with strong analytical background were lacking some biological background, so that some difficulty arose to comprehend PCR. For example, the most common misconception was that PCR is selective amplification of a specific region of DNA and not the complete DNA. Another misconception among students was that electrophoretic separation was dependent on charge only and independent of the size.

On the experimental side, the major challenge arose from the pipetting steps and small volumes employed. To facilitate these steps, the instructor should demonstrate all sample preparation steps first and have the students practice pipetting steps (as described in the Supporting Information). Another point to consider is sample contamination, which could falsify the results. Precautions, such as backup samples, instructor demonstrations, and aliquoting steps, are described in the instructor notes (see the Supporting Information) and were usually sufficient to avoid erroneous results.

■ ASSOCIATED CONTENT

Supporting Information

Instructor notes and student instructions. This material is available via the Internet at http://pubs.acs.org.

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JOURNAL OF CHEMICAL EDUCATION

LABORATORY EXPERIMENT

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Studies on the Food Additive Propyl Gallate: Synthesis, Structural Characterization, and Evaluation of the Antioxidant Activity

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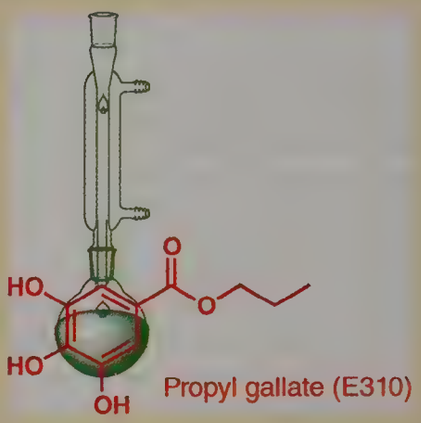
Supporting Information

ABSTRACT: Antioxidants are additives largely used in industry for delaying, retarding, or preventing the development of oxidative deterioration. Propyl gallate (E310) is a phenolic antioxidant extensively used in the food, cosmetics, and pharmaceutical industries. A series of lab experiments have been developed to teach students about the importance and significance of antioxidants in industry. In the first laboratory, the antioxidant propyl gallate is obtained and the structure identified. Students become acquainted with laboratory techniques such as extraction, crystallization, and thin-layer chromatography. In the second laboratory, spectroscopic data (IR, ^{1}H and ^{13}C NMR) is acquired and interpreted. Students become familiar with the basic concepts of organic compound identification. In the third laboratory, the antioxidant activity of the synthesized additive and gallic acid is evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay using trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as standard. Concepts such as free radical chemistry, preparation of analytical samples, calibration methods, and UV−vis spectrophotometry, are reviewed. This series of experiments can also be used to explore the effect of substituents on radical stability because structurally related compounds were found to have qualitatively different antioxidant profiles.



KEYWORDS: Second-Year Undergraduate, Analytical Chemistry, Interdisciplinary/Multidisciplinary, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Bioorganic Chemistry, Food Science, Free Radicals, Oxidation/Reduction

Food additives have been used since ancient times to preserve or increase the quality of food products.[1] A food additive is defined as "any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods".[2] Additives play a key role in maintaining the food quality and characteristics that consumers demand, keeping food safe, wholesome, and appealing from farm to fork. Food additives are divided into 25 groups and include flavorings, colorings, stabilizers (gelling and thickening agents), aroma and taste enhancers, sweeteners, and a wider range of preservatives and antioxidants. Food additives are carefully regulated and the general criteria for their use is that they must implement a useful purpose, must be safe, and do not mislead the consumer. To regulate the additives and inform consumers, each compound is named and assigned with a unique number. Initially, these were the "E numbers" used in Europe for all approved additives. This numbering scheme has now been adopted and extended by the Codex Alimentarius Commission to internationally identify all additives.

ANTIOXIDANTS

Oxidation−reduction reactions (redox reactions) are complementary chemical processes that involve loss of electrons (oxidation) by one reactant and a corresponding gain of electrons (reduction) by another reactant. Although oxidation reactions are crucial for life, they can also be damaging. Oxidation reactions happen when chemicals in the food are exposed to oxygen in the air. In natural conditions, animal and plant tissues contain their own endogenous antioxidants, but in foods, these natural systems break down and oxidation is bound to follow. Oxidation of food is a destructive process, causing loss of nutritional value and changes in chemical composition. Oxidation of fats and oils leads to rancidity and in fruits such as apples it can result in the formation of compounds that discolor the fruit. Fats and oils, or foods containing them, are the most likely to have problems with oxidation. Fats react with oxygen and even if a food has a low fat content it may still need the addition of an antioxidant.

Lipid oxidation processes can produce free radicals that are able to start radical chain reactions. The homolytic bond cleavage can be initiated by the action of external radical initiators such as heat, light, singlet oxygen, ionizing radiation, or by transition-metal catalysis involving copper, iron, or manganese ions. Air-tight packaging, using inert gases such as nitrogen, vacuum packing, and refrigeration can be used to delay the oxidation process. However, these processes can still be inefficient and

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adding antioxidants can be an effective way of extending the shelf life of a product.

Antioxidants are additives (classified from E300 to E385) defined as any substance, when present in low concentrations, that is capable of delaying, retarding, or preventing the development of deterioration due to oxidation. Antioxidants can inhibit or retard oxidation via two different mechanisms: by directly scavenging free radicals (primary antioxidant) or by a process that does not involve the direct scavenging of free radicals (secondary antioxidant).

Lipid oxidation reactions usually proceed by a three-stage free radical mechanism that includes initiation, propagation, and termination steps. In this process, primary radical-scavenging antioxidants (AH) can delay or inhibit the initiation step by neutralizing a lipid radical, $L^{\bullet}$ (eq 1), or inhibit the propagation step by reacting with a peroxyl radical, $LOO^{\bullet}$ (eq 2), or alkoxyl radical, $LO^{\bullet}$ (eq 3):

$$L^{\bullet} + AH \rightarrow LH + A^{\bullet} \quad (1)$$

$$LOO^{\bullet} + AH \rightarrow LOOH + A^{\bullet} \quad (2)$$

$$LO^{\bullet} + AH \rightarrow LOH + A^{\bullet} \quad (3)$$

Termination reactions, in which free radicals combine to form molecules that do not feed the propagating reactions, will stop the self-propagating chain. Different products formed between the antioxidant free radical ($A^{\bullet}$) and lipidic radicals can also be obtained in the process:

$$A^{\bullet} + LOO^{\bullet} \rightarrow LOOA \quad (4)$$

$$A^{\bullet} + LO^{\bullet} \rightarrow LOA \quad (5)$$

If there is an increase in the A−H and L−H bond dissociation energies, the activation energy of the antioxidant reactions increases. Hence, the efficiency of the antioxidant increases with decreasing A−H bond strength. In other words, the weaker A−H bonds yields the more efficient antioxidants. Secondary antioxidants operate by a variety of mechanisms such as binding to transition-metal ions or deactivating singlet oxygen. However, some antioxidants operate by both ways. Synergism between antioxidants has also been noted, and many commercial antioxidant formulations contain several antioxidants.

Antioxidants are widely used in baked foods, cereals, fats, oils, soaps, medicines, and cosmetics. The major antioxidants of organic type are

- Tocopherols (E306−E309), BHA (butylhydroxyanisole, E320) and BHT (butylhydroxytoluene, E321); gallic acid derivatives (E310−E312) are regularly applied to protect edible fats, vegetable oils, and salad dressings from turning rancid.
- Ascorbic acid and derivatives (E300−E304) and citric acid (E330) are usually used to preserve the color of freshly cut fruits and vegetables.

Chemically, gallates are alkyl esters of the 3,4,5-trihydroxybenzoic acid (gallic acid) and differ from each other in their side chains. The variants usually employed are propyl gallate (PG), octyl gallate (OG), and dodecyl gallate (DG). They have been extensively used in the food, cosmetics, and pharmaceutical industries.[3] According to the European Directive on alimentary additives,[4] the maximum allowed dose is 200 mg/kg in fats and oils destined to the professional manufacture of heat-treated alimentary products and in a number of manufactured foods, with the exceptions of dehydrated potatoes (25 mg/kg), chewing gums (400 mg/kg), and dietary supplements (400 mg/kg).

■ ANTIOXIDANT CAPACITY ASSAYS

The potency of antioxidants under real circumstances can be investigated in an in vitro model system with relatively simple and controlled circumstances. Although in vitro methods provide a useful indication of antioxidant activities, data obtained by these methods are difficult to apply to biological systems. The most widely employed chemical tests have different assessment end points such as the radical scavenging capacity, the uptake of oxygen, the inhibition of induced lipid autoxidation, the reducing power, and the chelation of the transition metals. Unfortunately, none of these assays were regarded as universal.[5−11] Two types of analytical methods are currently used for evaluation of the antioxidant activity: (i) inhibition methods, in which the inhibition of oxidative damage of the target molecule is measured in the presence of antioxidants and (ii) methods based on direct measurement of stable free radicals scavenging by antioxidants present in the sample. One of the most popular and simple spectrophotometric methods to measure the ability of antioxidants to trap free radicals is the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.[5−11] It is a rapid, simple, inexpensive, and widely used method to measure the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity of foods. It can also be used to quantify antioxidants in complex biological systems for solid or liquid samples.[12]

■ EXPERIMENT OVERVIEW

A series of lab experiments have been developed to teach students about the importance and significance of antioxidants in industry. In the first laboratory, the antioxidant propyl gallate (E310) is obtained and the structure identified. Students become acquainted with laboratory techniques such as extraction, crystallization, and thin-layer chromatography (TLC). In the second laboratory, spectroscopic data (IR, ^{1}H NMR, and ^{13}C NMR) are acquired and interpreted. Students become familiar with the basic concepts of organic compound identification. In the third laboratory, the antioxidant activity of the synthesized additive and gallic acid is evaluated by DPPH assay using trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as standard.

These experiments were devised for chemistry-based curriculums, namely, chemistry, food chemistry, and chemical engineering courses. All the students had taken general chemistry, organic, and analytical chemistry disciplines. Students performed these experiments individually or in groups of two or three students. Each group of students or an individual student submits a lab report one week after completing the lab work.

Synthesis and Structural Characterization of Propyl Gallate (E310)

Phenolic antioxidants (ArOH) can act as free radical scavengers. The activity depends mainly on different structural features such as O−H bound dissociation energy, resonance delocalization of the phenoxyl radical ($ArO^{\bullet}$), and steric hindrance derived from the presence of bulky substituents in the aromatic ring. The chemical modification of phenolic acid antioxidants, for instance, by esterification with *n*-alkyl alcohols, has been suggested as a

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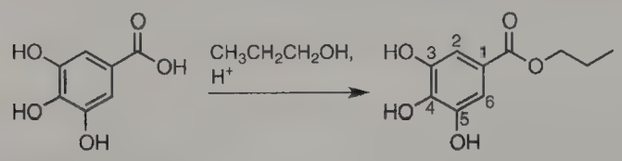
Scheme 1. Synthesis of Propyl Gallate by Fischer Esterification



gallic acid (E300) propyl gallate (E310)

suitable procedure to enhance their hydrophobicity and improve their antioxidant properties.

Esters are a common derivative of carboxylic acids and are widely distributed in both nature and industry. They represent important final products or synthetic intermediates for food, pharmaceutical, and cosmetic industries. A classic procedure to synthesize esters is the Fischer esterification wherein a carboxylic acid is treated with an alcohol in the presence of a mineral inorganic acid catalyst.[13] In this condensation reaction, the equilibrium may be influenced by either removing one product from the reaction mixture or by employing an excess of one reactant. The reaction proceeds by a nucleophilic acyl substitution mechanism. The reaction product is purified by multiple liquid–liquid extraction and crystallization with a mixture of solvents. The product is identified by the melting point and infrared spectra. Nuclear magnetic resonance spectra are also used for further practice in interpreting the data.

Evaluation of the Antioxidant Activity

The DPPH method is used in the quantification of free radical scavenging activity. The process involves a color change from violet to yellow that can be easily monitored at 515–520 nm. The DPPH changes color when the nitrogen atom in DPPH is reduced by a process in which a hydrogen atom from antioxidant compounds plays a part. The DPPH method is simple and only requires a UV–vis spectrophotometer: in the presence of a hydrogen or electron donor (free radical scavenging antioxidant), the absorption intensity decreases, and the radical solution is discolored according to the number of electrons captured. There are different methods of interpreting the results of the DPPH assay.[12] The majority of the studies express the results as the IC_{50} value defined as the quantity of antioxidant necessary to decrease the initial DPPH concentration by 50%. This value is calculated by plotting inhibition percentage against extract concentration. Other indexes express the results as the antioxidant activity power, namely, the antiradical power (ARP), that is defined as $1/EC_{50}$ (where EC is the efficiency concentration; the larger the ARP, the more efficient the antioxidant) or the antioxidant activity index (AAI) calculated as AAI = final DPPH concentration/IC_{50}.

A comparative study of propyl gallate, gallic acid, and trolox (the water-soluble vitamin E analogue) is carried out to evaluate the relative antioxidant activities. This assessment evaluates the influence of the alkyl side chain in the stabilization of the radical formed during oxidation and the influence of hydrophobicity in the improvement of the antioxidant properties.

■ EXPERIMENTAL PROCEDURE

First Class Period: Synthesis and Purification of Propyl Gallate (E310)

Propyl gallate is synthesized by a Fisher esterification reaction (Scheme 1). The analytical control is performed by TLC using the following system: silica gel, dichloromethane/methanol (8:2). The spots are visualized under UV detection (254 and 366 nm) and iodine vapor. The purification steps include a multiple liquid–liquid extraction and recrystallization.

Second Class Period: Structural Characterization Propyl Gallate

The melting point of propyl gallate is determined to assess its purity. Students obtain infrared spectra using potassium bromide disks and the most significant absorption bands are reported (υ_{max}, cm^{-1}). Students acquire the ^{1}H NMR and ^{13}C NMR spectra at room temperature with dimethylsulfoxide-d_6 as the solvent; the chemical shifts, expressed in δ (ppm) values, and coupling constants (J) are reported.

Third Class Period: DPPH Radical Scavenging Assay

The antioxidant activity of gallic acid (GA), propyl gallate (PG), and trolox are determined using the DPPH method. Solutions of DPPH and antioxidants at different concentrations are prepared in ethanol. After an incubation period at room temperature, in the dark, the absorbance of the solutions at 517 nm is measured using a calibrated UV–vis spectrophotometer. IC_{50}, antiradical power (ARP), and antioxidant activity index (AAI) are calculated and critically discussed.[5,9]

■ HAZARDS

Protective clothing, goggles, and gloves should be worn. Gallic acid is hazardous in case of eye contact and ingestion and slightly hazardous in the case of skin contact and inhalation. Propyl gallate may induce skin sensitization. DPPH is classified as harmful and a sensitizer. It can cause skin, mucous, and eye irritation. Dichloromethane is harmful if swallowed or inhaled; may be harmful by skin contact. Additional information regarding the potential hazards in handling these chemicals are available in the Supporting Information.

■ DISCUSSION

The main structural feature responsible for the antioxidant and free radical-scavenging activity of propyl gallate (PG) and gallic acid (GA) is the number and arrangement of phenolic groups in the aromatic nucleus. This type of antioxidant is able to donate the hydrogen atom of the phenolic group to free radicals, thus, stopping or minimizing the propagation radical chain during the oxidation process. The electron-withdrawing property of the carboxylic group has a negative influence on the H-donating ability of the hydroxybenzoic acids. To minimize this drawback, this group is often esterified with fatty alcohols and, in some cases, a positive impact on the resulting antioxidant activity is attained. Moreover, the relative low solubility of phenolic compounds in apolar media, seen as a disadvantage considering their use as antioxidants on organic media, could be overcome by increasing their hydrophobicity via reactions that increase lipophilicity (e.g., esterification).

DPPH assays constitute a widespread and easy-to-use protocol to study antiradical reactivity. Accordingly, the antioxidant activities of PG, GA, and the reference compound trolox are evaluated by their effects of scavenging the stable free radical, 1,1-diphenyl-2-picryl hydrazyl (DPPH). The data obtained show that GA has a lower DPPH radical-scavenging activity than its ester derivative, PG. The different indexes considered for the potency expression of the antioxidant activity, namely, the antiradical power (ARP) and the antioxidant activity index

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(AAI), strengthen this trend. According to the AAI index, it is possible to conclude that all the compounds under study exert a noteworthy antioxidant activity. PG emerges as the most effective of the tested compounds and much more efficient than the trolox standard used as reference. This behavior is consistent with the antiradical activity described in literature for related phenolic systems of ester type.[10]

■ CONCLUSION

In addition to motivating class enthusiasm toward synthetic organic and analytical chemistry, this experiment encompasses educational practical and theoretical concepts. Students become reacquainted with laboratory techniques such as heating under reflux, extraction, evaporation, crystallization, vacuum filtration, and thin-layer chromatography. The experiment allows students to collect and interpret their own spectroscopic data (IR, ^{1}H NMR, and ^{13}C NMR) obtaining the basic concepts of identification of organic compounds. The knowledge gained by analyzing their own data allows students to evaluate their work. Of special interest is the ^{1}H NMR spectra that illustrates proton shielding−deshielding and spin−spin splitting. Purity evaluation was also evaluated by chromatographic and melting point measurements. The evaluation of the antioxidant activity of the propyl gallate is proposed as an opportunity for the students to criticize their own work checking the properties of the synthesized compound and also to review several concepts of the utmost importance in food chemistry.

■ ASSOCIATED CONTENT

Supporting Information

Instructions for the students; notes for the instructor; spectra of gallic acid and propyl gallate for using in classroom for those without direct student access to the apparatus. This material is available via the Internet at http://pubs.acs.org.

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LABORATORY EXPERIMENT

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Nature's Migraine Treatment: Isolation and Structure Elucidation of Parthenolide from *Tanacetum parthenium*

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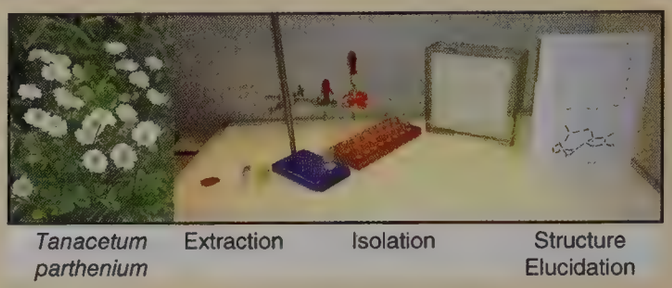
Supporting Information

ABSTRACT: The purpose of this experiment is to provide students with the essential skills and knowledge required to perform the extraction, isolation, and structural elucidation of parthenolide from *Tanacetum parthenium* or feverfew. Students are introduced to a background of the traditional medicinal uses of parthenolide, while more modern applications of parthenolide are also presented. Clinical data supporting the use of feverfew in the treatment of migraine is presented. Methods outlining the accurate extraction and isolation of parthenolide from the powdered, dried flowering tops of feverfew are described. The experiment allows students to acquire and use such skills as extraction, flash column chromatography, and thin-layer chromatography. Structural elucidation is carried out on parthenolide using techniques such as infrared (IR) spectroscopy, high-resolution mass spectrometry (HRMS), and nuclear magnetic resonance (NMR) spectroscopy. Nuclear Overhauser enhancement spectroscopy (NOESY) and X-ray crystallography are employed to establish the three-dimensional conformation of the structure. The student can isolate parthenolide with an approximate yield of 0.2% and the experiment can be completed over two 3-h laboratory sessions. Finally, questions are provided in the student handout, requiring that students engage further in topics associated with the context of this practical.



KEYWORDS: Graduate Education/Research, Upper-Division Undergraduate, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Medicinal Chemistry, NMR Spectroscopy, Natural Products, Thin Layer Chromatography, X-ray Crystallography

Many natural plant products have been used in the treatment of a wide variety of ailments and disease since ancient times. This experiment imparts a valuable learning experience to the students in the use of *Tanacetum parthenium* (Figure 1) as a herbal medicine and the application of important analytical techniques commonly used in the laboratory for extraction and isolation purposes. The principal isolation technique employed in this practical is flash column chromatography. Widespread use of flash column chromatography in research fields such as anti-inflammatory,[1] anti-allergic,[2] and antimalarial[3] drug design programs underpins its status as a valuable analytical technique. This experiment represents an effective educational tool in column chromatography, offering students the opportunity to perform and thoroughly master the skills required to achieve precise isolation of a substance of interest from a complex mixture. Students also utilize high-resolution mass spectrometry (HRMS), infrared (IR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy as a means to performing a complete structural elucidation study on parthenolide. Work on this project complements our previous developments in phytochemical isolation, including valtrate from *Centranthus ruber*,[4] galantamine from *Leucojum aestivum*,[5] as well as other laboratory-based experiments such as betulin from birch bark[6] and monoterpenes from spearmint.[7]

BACKGROUND

Feverfew is a member of the Asteraceae (Compositae) family and has been classified as *T. parthenium*, *Chrysanthemum parthenium*, or *Leucanthemum parthenium*, with *T. parthenium* being the most favored classification.[8] Feverfew has long been referred to as "a medieval aspirin"[9] and was used to a treat a broad spectrum of disorders including fever, headache and migraine, menstrual problems, and childbirth difficulties as well as stomach ache, toothache, and insect bites.[10]

A surge in scientific interest in this plant during the 1980s led to the undertaking of a number of clinical trials assessing the effects of *T. parthenium* in the prophylaxis of migraine.[11−14] More recently, it has been shown that parthenolide exhibits anti-inflammatory properties[15,16] and antileishmanial activity,[17] as well as chemopreventative properties[18] and antitumor activity.[19]

EXPERIMENTAL OVERVIEW

This experiment is aimed at fourth-year undergraduate or first-year postgraduate students who have a comprehensive understanding of the spectroscopic techniques used in structure

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Figure 1. Photograph of *T. parthenium* taken from author's (J.J.W.) garden.

elucidation of organic compounds. It has been completed with consistency and reproducibility by students in a masters-level pharmaceutical analysis course. The plant material is readily available and was collected from the author's (J.J.W.) garden. The experiment affords the student the opportunity to follow the entire procedure of parthenolide isolation, from its extraction from the flowering tops of *T. parthenium* to elucidation of its structure using spectroscopy. It places particular emphasis on the importance of flash column chromatography for the isolation of phytochemicals in pure form from complex mixture and the use of modern spectroscopic techniques for the identification of natural products.[20] The students are not informed of the structure of the compound, which is of moderate complexity, therefore, providing a challenging exercise for an upper undergraduate or graduate student. A variety of one- and two-dimensional NMR spectra, as well as IR and mass spectra, are provided in the Supporting Information. The NMR data obtained on parthenolide include ^{1}H NMR and ^{13}C NMR spectra, in addition to proton−proton correlated spectroscopy (HH−COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond coherence (HMBC). IR and MS spectral evidence support the information gained from these spectra. Students also utilize X-ray crystallography and nuclear Overhauser effect spectroscopy (NOESY) to identify the exact stereochemical conformation of parthenolide.

■ EXPERIMENTAL DETAILS

This laboratory was divided into three sections: (i) crude product extraction and isolation, (ii) chromatography (flash column and TLC), and (iii) spectroscopy (MS, IR, and NMR). In week one of the practical, approximately 1 mg of parthenolide was generated from 0.5 g of the powdered flowering tops of *T. parthenium*. The process involved mixing the finely powdered plant material in 5 mL of dichloromethane for 20 min using a magnetic stirrer. The mixture was filtered and reduced in volume to allow for easy transfer onto the flash column. A standard Pasteur pipet of internal diameter of 5 mm was used to prepare the column (see student handout in Supporting Information for precise details of column preparation). The solvent system of choice for the flash chromatography was hexane/ethyl acetate, for which the following gradient system was used to achieve optimal resolution; hexane/ethyl acetate 9:1 (9 mL/1 mL), 6:1 (6 mL/1 mL), 5:1 (5 mL/1 mL), and 4:1 (12 mL/3 mL). Fractions of approximately 0.5 mL in volume were obtained. The first 10 mL of mobile phase was run through the column and the eluent was collected. For the remaining 10 mL of solvent, 20 fractions were obtained in numbered glass sample vials. Fractions containing pure parthenolide were identified by TLC by comparison with a parthenolide standard, using hexane/ethyl acetate (3:1) as the solvent system. The appropriate fractions were combined and solvent was removed using a rotary evaporator. The yield of pure parthenolide was recorded. Crystals suitable for X-ray analysis were slowly grown from a methanol solution after pooling the isolated compound samples from five students.

■ HAZARDS

This experiment involves the use of some potentially toxic substances including dichloromethane, hexane/ethyl acetate, silica gel, and vanillin spray reagent. Dichloromethane is a carcinogen, is toxic by inhalation, and causes irritation and burning pain on prolonged contact. Hexane is a flammable liquid and vapor, is harmful or fatal if swallowed, and causes irritation to skin, eyes, and respiratory tract. Ethyl acetate is flammable and harmful if inhaled and slightly hazardous in case or skin or eye contact. Exercise great care when handling these substances and use in a fume hood. Laboratory coats and suitable eye protection must be worn while undertaking this experiment. Parthenolide is a known skin irritant. Protective gloves must be worn throughout the practical.

■ RESULTS AND DISCUSSION

IR, NMR, and HRMS spectra were obtained on the substance isolated to fully elucidate its structure. A strong absorption band on the IR spectrum at 1756 cm^{-1} is indicative of an ester group, whereas the band at 940 cm^{-1} may suggest the presence of an epoxide. The weak absorption band at 1654 cm^{-1} indicates alkene functionality. HRMS provides a molecular ion peak with an m/z value of 271.1306. Taking into account the addition of a sodium adduct, a possible molecular weight of approximately 248 (271.1306 − 23) for the structure is a reasonable assumption. A peak representing an ion with an m/z value of 248.1646 justifies this. Referring to the ^{13}C NMR spectrum, 15 carbon signals are evident, perhaps representative of a sesquiterpene-like compound, whereas a total proton count of 20 is provided by the ^{1}H NMR spectrum. Further analysis of the ^{13}C and DEPT spectra supplies evidence of two CH_3, five CH_2, and four CH signals in addition to four quaternary carbon atoms. It may be deduced that, of the 15 signals present in the ^{13}C NMR spectrum, one represents the carbonyl carbon of a conjugated ester, four are indicative of olefinic carbons, and three peaks signify carbons with a carbon−oxygen single bond. Knowing the molecular weight of the compound to be 248.1646 and having proven the existence of 15 carbons and 20 hydrogens, three oxygen atoms must be present, therefore, deriving an empirical formula of $C_{15}H_{20}O_3$. Further evidence to substantiate this formula is provided with data accompanying the mass spectrum, which generates a range of molecular compositions with a possible molecular weight of approximately 248. Of the 10 possible options, only one molecular formula contains the 15 required carbon atoms that correspond with the suggested empirical formula (see instructor's notes in the Supporting Information).

Calculation of the index of hydrogen deficiency yields an unsaturation index of 6. Already established is the presence of

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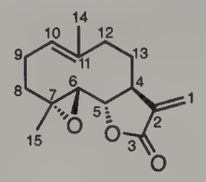

Figure 2. Parthenolide.

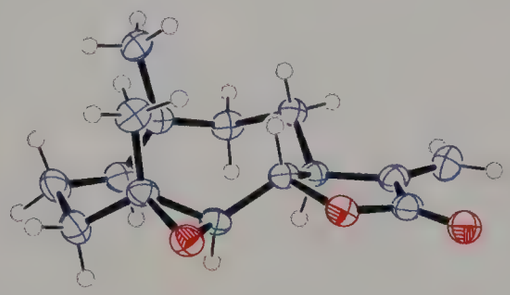

Figure 3. Ortep presentation of parthenolide generated by X-ray crystallography The X-ray crystal structure obtained was identical to that previously published.[21]

two C=C double bonds and one carbonyl group, suggesting ring formations to account for the remaining three unsaturation equivalents. In the IR spectrum, the C=O stretching vibrations at 1756 cm^{-1} is strongly indicative of an α, β-unsaturated γ-lactone functionality. In addition, the mass spectrum illustrates fragmentation of the structure with a loss of CO_2, resulting in a daughter ion peak with an m/z value of 227.1404. Consideration of these features, coupled with application of the unsaturation equivalent rule, leads to an assumption that a lactone ring is present. Resonances for two carbon atoms in the chemical shift region of the ^{13}C NMR spectrum consistent with a C−O single bond, together with one unassigned oxygen atom, suggests ether or possibly epoxide functionality. IR spectral evidence of a ring deformation band at 984 cm^{-1} is consistent with the presence of an epoxide. Combining this evidence with an unsaturation equivalent establishes with high probability the presence of an epoxide. The one remaining unsaturation index must be due to an additional ring formation. Therefore, the suggestion of a sesquiterpene lactone with epoxide functionality as a possible structure is acceptable.

Imperative to complete elucidation of the structure is detailed use of 1-D and 2-D NMR spectroscopic data. Using HH−COSY, HMQC, and HMBC spectra, in conjunction with ^{1}H NMR and ^{13}C NMR spectra, the C−H framework of the molecule is assigned. One approach to the elucidation of the structure, shown in Figure 2, is outlined in the Supporting Information. With assignment of the structure complete, the conformation of the molecule requires clarification. A three-dimensional physical model of the structure was constructed. Manipulation of the model yielded two possible structural configurations, differing in the arrangement of the two CH_3 (C14 and C15) groups above and one each above and below the plane.

A conformation whereby both methyl groups are pointing upward was deduced. NOE spectral studies and X-ray crystallographic analysis (Figure 3) indicate that the molecular framework is restricted to a single conformation. Strong contours indicating through-space coupling of the two methyl group protons (H14 and H15) establish that both groups are situated above the plane of the ring. Very strong, through space, coupling is observed between H5 and H15 with medium coupling detected between H5 and H14, suggesting that H5, resonating as an overlapping double doublet with identical J values (9.0 Hz) is α oriented. The large coupling constant observed for H5 suggests that it is antiperiplanar to H4 and H6. The configuration of the C10−C11 double bond is established due to lacking evidence indicating correlation of H10 with H15. This suggests that these protons are located on opposite planes of the double bond, thus, representing H10 as β oriented and an E configuration for the double bond. Strong correlation between H10 and H6 establishes that H6 resides below the plane of the ring.

■ ASSOCIATED CONTENT

Supporting Information

Student handout; instructor's notes; spectra. This material is available via the Internet at http://pubs.acs.org.

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Nature's Cholesterol-Lowering Drug: Isolation and Structure Elucidation of Lovastatin from Red Yeast Rice-Containing Dietary Supplements

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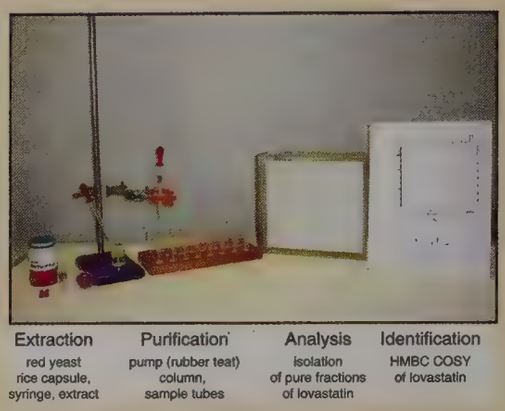
Supporting Information

ABSTRACT: Red yeast rice, produced by fermenting the fungus, *Monascus purpureus*, on rice (*Oryza sativa* L. gramineae), is commonly used as a dietary supplement. It contains lovastatin, a member of the statin family of compounds, and is licensed for use as a cholesterol-lowering agent. This experiment involves the isolation and structure elucidation of lovastatin from red yeast rice-containing dietary supplements. Isolation of the neutral constituents in red yeast rice can be performed using either ether or ethyl acetate as extracting solvent, and flash column chromatography combined with thin-layer chromatography is used to isolate lovastatin in pure form from the extract. During the course of the experiment, the students isolate lovastatin with an average recovery of 0.31%. IR, MS, and detailed NMR spectroscopic analyses confirm the structure of lovastatin. The experiment can be completed over two, 3-h laboratory sessions and is suitable for students at the third- or fourth-year undergraduate level. Overall the experiment acquaints the student with the techniques required to isolate and complete structure elucidation studies on natural products. This experiment also highlights that dietary supplements may contain substances with potent pharmacological actions that are not expected from a cursory inspection of the labeled products.



KEYWORDS: Upper-Division Undergraduate, Laboratory Instruction, Organic Chemistry, Problem Solving/Decision Making, Chromatography, Drugs/Pharmaceuticals, IR Spectroscopy, Mass Spectrometry, NMR Spectroscopy, Natural Products

A historical overview of red yeast rice (*Monascus purpureus*) development as a dietary supplement is presented to the students. Students then isolate and purify lovastatin (Figure 1) from red yeast rice and use 1- and 2D NMR, IR, and MS spectroscopy for structural elucidation. The experiment complements other laboratory-based experiments on the isolation of natural products including valtrate from *Centranthus ruber*,[1] galantamine from *Leucojum aestivum*,[2] betulin from birch bark,[3] monoterpenes from spearmint,[4] shikimic acid from star aniseed,[5] curcumin from turmeric,[6] and thiarubrine A from *Ambrosia artemisiifolia*.[7]

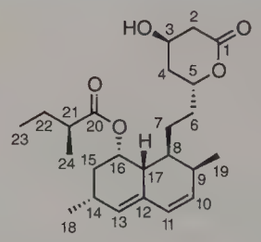


Figure 1. The structure of lovastatin.

BACKGROUND

Red yeast rice, otherwise known as red koji or hong qu, is prepared by fermenting a fungal strain identified as *M. purpureus* on steamed rice that produces the secondary metabolite, lovastatin. The medicinal and culinary values of red koji have been documented as far back as 800 C.E. Red yeast rice is said to promote food digestion and blood circulation. A prominent pharmacologist of the Ming dynasty (1368–1644), Li Shizen, believed that hong qu enhances "digestion and blood circulation, can strengthen the spleen and dry the stomach,"[8] and Miao Xiyong, another important pharmacologist from the Ming dynasty described hong qu, according to the Ying system, as having a beneficial effect on spleen and stomach. This ancient Chinese medical theory relating to the blood circulation is based on the belief that blood behaves by the rule of *like attracting like*.[8] Lovastatin, also known as mevinolin or monacolin K, is the primary active constituent of red koji. It belongs to the drug class of statins, which are hypolipidemic agents used in the treatment of hypercholesterolemia, thus minimizing the risk of cardiovascular disease. Lovastatin works by reversible competitive inhibition of a key rate-limiting enzyme in cholesterol synthesis called 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. The discovery that lovastatin acts as a lipid-lowering agent led to investigations of its effectiveness in reducing low-density lipoprotein cholesterol (LDL-C) termed the "bad" cholesterol. In the Air Force–Texas Coronary Atherosclerosis Prevention Study, the effect of lovastatin to treat

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hypercholesterolemia was evaluated in a double-blind, placebo-controlled trial that showed that lovastatin is significantly better than placebo at preventing coronary disease.[9] Lovastatin is currently marketed as Mevacor, Altocor (now Altoprev in the United States), and Advicor. It is also found in dietary supplements containing red yeast rice preparations.

■ EXPERIMENTAL OVERVIEW

The laboratory is particularly suited to third- or fourth-year undergraduate students who have a basic understanding of the chromatographic and spectroscopic techniques used in the identification of natural or synthetic compounds. It can be conducted over two, 3-h laboratory sessions. Students from many different disciplines including pharmacy, medicine, medicinal chemistry, and organic chemistry will find this experiment interesting and informative. In the introductory part of the experiment, the possible pharmacological properties of dietary substances are highlighted. In this example, the constituent of interest, lovastatin, is widely prescribed as a cholesterol-lowering agent. The experiment examines the entire procedure for the isolation and structure elucidation of lovastatin from red yeast rice, including extraction from the raw material, purification, and spectroscopic characterization. Throughout the experiment, students practice various techniques involved in the extraction and isolation of medicinally important natural products. These include the use of the correct solvent to attain a lovastatin-rich fraction from a complex matrix as well as thin-layer and flash column chromatographic techniques used in the isolation of lovastatin from the prepared extract. Students are required to think critically about how to elucidate the structure of lovastatin through the interpretation of 1- and 2D NMR spectroscopic data. The NMR spectroscopic methods include 1D ^{1}H and ^{13}C spectra as well as 2D proton−proton (H−H), proton−carbon (HMQC), and long-range proton−carbon correlation (HMBC) techniques. In addition, students learn how to interpret infrared (IR) and mass spectral data to strengthen the information gathered from the 1- and 2D NMR spectra.

In addition, students are assigned a topic and asked to present a PowerPoint slide(s) on an aspect relating to the "bench-to-bedside" development of lovastatin as a cholesterol-lowering drug. This interactive seminar occurs in the second session. As a resource for the slide preparation, the students are provided with the full text of this article, the student handout, and instructor notes (see the Supporting Information).

■ EXPERIMENTAL SECTION

In the first session of the experiment, the content of two capsules of a red yeast rice-containing dietary supplement (~980 mg) (Napiers) was vigorously shaken with ether (4 mL) for 15 min, taken up into a syringe (5 mL), and filtered through a 0.2 μm filter. The insoluble matrix was washed with ether (2 × 3 mL). The combined filtrate was reduced to dryness, and reconstituted in minimum volume of dichloromethane for transfer onto the flash column. The flash column was prepared from a standard 150 mm long Pasteur pipet. The base of the pipet was plugged with cotton wool and protected with sodium chloride to a depth of 5 mm. The column was filled with flash silica (particle size range 0.043−0.062 mm) to a depth of 55 mm and protected at the top with a 5 mm layer of sodium chloride (a diagram is in the Supporting Information). Hexane was used to pack the column. The mobile phase was a gradient system consisting of hexane (1 mL), hexane/ethyl acetate (2:1, 6 mL), and hexane/ethyl acetate (1:1, 8 mL). The volume of eluent collected in each fraction was approximately 0.5 mL. The fractions containing pure lovastatin were identified using silica gel thin-layer chromatography (TLC) employing hexane/ethyl acetate (1:1) as mobile phase, for which lovastatin has an R_f of 0.24. The yield of pure lovastatin was calculated. Overall, this technique is reproducible but requires the student to use the correct quantity of silica and the minimum volume of dichloromethane.

In the second session, IR, mass spectrometry, and detailed NMR spectroscopic analyses confirmed that the substance isolated was lovastatin. The most noticeable features in the IR spectrum of lovastatin were the broad hydroxyl peak at 3400 cm^{-1}, the aliphatic and vinylic C−H stretching peaks between 2900 and 3000 cm^{-1}, and the peak at 1724.25 cm^{-1} for the two carbonyl ester groups. The high-resolution mass spectrum (HRMS) spectrum revealed the $(M + Na)^+$ at m/z 427.2444 as the most abundant ion, whereas loss of the hydroxyl group is evident by the fragment ion at m/z 410.2474. Simple direct comparison of each of the 1D ^{13}C NMR spectra revealed that lovastatin contains 24 carbons (4 × CH_3, 6 × CH_2, 11 × CH, and 3 × C). The 2D spectra were particularly useful when trying to assign the resonance positions of the proton and carbon signals. The most useful starting point was the carbonyl at position 1 (Figure 1). Its resonance position could easily be distinguished from that of the other carbonyl at position 20 as it only couples to methylene and methine protons in the HMBC spectrum. This spectrum could then be used to find the precise location of the protons on C2 and C3 whereas the HMQC spectrum enabled identification of C2 and C3. The use of the H−H COSY and HMQC spectra allowed for the determination of the resonance position of the ^{1}H and ^{13}C signals at positions C4, C5, and C6. Knowing the location of C6 and using a combination of HMBC and HMQC spectra enabled identification of the ^{1}H and ^{13}C signals at positions C7 and C8. From here, sequential use of the H−H COSY and HMQC spectra allowed for the identification of the methine, vinyl, or methylene signals at positions C9−C11 and C13−C19. The resonance position of the alkene carbon at position C12 was easily identified as it is the only quaternary carbon in this region of the spectrum. In the HMBC spectrum, the C20 carbonyl showed long-range coupling to the protons on C21, C22, and C24. The use of the HMQC spectrum allowed for the determination of their carbon signals leaving only the CH_3 at position C23 to be identified through the use of the HMQC spectrum. Complete assignment of all signals is provided in the Supporting Information.

■ HAZARDS

The experiment involves the use of some potentially hazardous reagents and flammable solvents. Dichloromethane, hexane, ethyl acetate, ether, vanillin, and sulfuric acid are toxic if inhaled, swallowed, or absorbed through the skin. Care must be taken when handling these and they must be used in the fume hood. Contact with skin and clothes should be avoided. Silica gel is a known carcinogen; thus, it should be used in the fume hood and handled with due care and attention. Laboratory coat and eye protection must be worn throughout the experiment. Latex gloves should also be worn when handling the vanillin spray reagent.

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■ SUMMARY

The laboratory was performed with consistent reproducibility by 16 students from the third-year pharmacy class. During the course of the laboratory, the students were exposed to a simple, yet robust, technique for the isolation in pure form of a medicinally important natural product from a complex mixture. They gained appreciation of the importance of the correct choice of gradient mobile-phase system for the precise isolation of lovastatin from the crude extract. Typically, the students were able to isolate between 2 and 3 mg of lovastatin from two capsules. Working with the instructor, confirmation of the identity of the compound through spectroscopic techniques was viewed by the students as an important example of problem-based learning. The compound confirmation was intense and required the students to freely move between the various spectra to fully assign all of the important signals. Overall, the students were positive about the experiment as they could clearly see how the techniques used could also be applied to the isolation and structure elucidation of other examples of natural products including galantamine from *L. aestivum* and valtrate from *C. ruber*.

■ ASSOCIATED CONTENT

Supporting Information

A student handout, including questions; notes for the instructor, including answers to the student questions; spectra; tabulated spectral data. This material is available via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

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LABORATORY EXPERIMENT

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A Safer and Convenient Synthesis of Sulfathiazole for Undergraduate Organic and Medicinal Chemistry Classes

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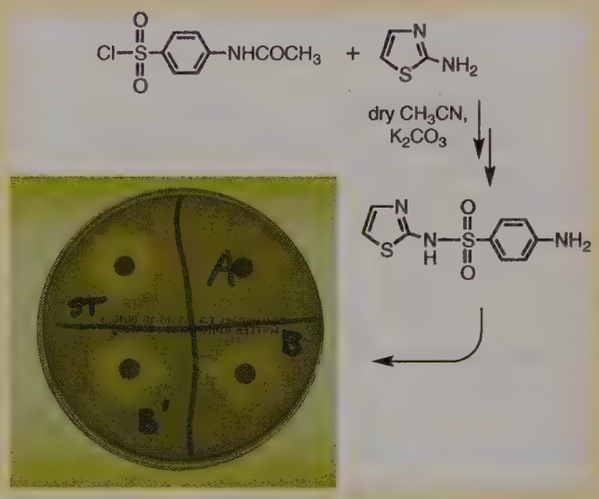
Supporting Information

ABSTRACT: A safer method for the synthesis of the sulfonamide drug sulfathiazole, for undergraduate classes, is described. This method improves upon procedures currently followed in several undergraduate teaching laboratories for the synthesis of sulfathiazole. Key features of this procedure include the total exclusion of pyridine, which has potential health hazards, from the first step of the synthesis and a simplified procedure for converting the intermediate *p*-acetamidobenzenesulfonamide to the final product by acid hydrolysis. Characterization of the synthetic sulfathiazole obtained by the modified route was achieved using MS, NMR, and antibacterial activity testing of the synthetic product against *Escherichia coli* DH5α.

KEYWORDS: Upper-Division Undergraduate, Biochemistry, Laboratory Instruction, Organic Chemistry, Safety/Hazards, Hands-On Learning/Manipulatives, Bioorganic Chemistry, Drugs/Pharmaceuticals, Medicinal Chemistry, NMR Spectroscopy



The discovery of Prontosil by Gerhard Domagk in 1932 marked the beginning of the modern chemotherapeutic era.[1−3] Since then, over 5000 sulfonamides have been prepared and tested for the prevention and cure of bacterial infections in humans.[4] Synthesis of the sulphonamide drug sulfathiazole has been part of the medicinal chemistry course since 2002. This experiment is executed in two laboratory sessions (6 h in total). Students complete the synthetic part of sulfathiazole in the first session. In the second session, students purify the crude product by recrystallization, record IR spectra, and carry out antibacterial testing of the purified product against *Escherichia coli*. This laboratory is also appropriate for an organic or biochemistry laboratory.

OLD SYNTHETIC SCHEME

The synthetic procedure based on available literature is shown in Scheme 1.[5] In this procedure, 4-acetamidobenzenesulfonyl chloride **1** is slowly added with stirring to a solution of 2-aminothiazole **2** dissolved in dry pyridine. The reaction mixture, after refluxing for 25 min, is poured into ice and the intermediate *p*-acetamidobenzenesulfonamide **3**, which precipitates out, is collected by vacuum filtration. Intermediate **3** is then subjected to hydrolysis using 2 M NaOH for 50 min. The reaction mixture is neutralized carefully with hydrochloric acid to precipitate the product sulfathiazole **4**, is collected by vacuum filtration, and is recrystallized from hot ethanol.

One major drawback of this procedure is the use of pyridine during the first step of the synthesis. Pyridine is a toxic compound with an unpleasant odor and can enter the body by inhalation or ingestion causing nausea, insomnia, headache, and abdominal pain.[6] Pyridine and some of its derivatives are also known to induce infertility in male rats and is considered as a potential carcinogen.[7−11] The quantity of pyridine used in this experiment has been minimal such that the health and safety regulation limits are complied with. However, given the potential health concerns stated above, an attractive alternative for large classes would be to exclude pyridine from this experiment.

Recovery of the product sulfathiazole **4** from the reaction mixture after alkaline hydrolysis of intermediate **3** posed another practical difficulty for students. In the original procedure, shown in Scheme 1, sulfathiazole **4** was precipitated out of the alkaline reaction mixture by optimizing the pH to near neutral using a combination of concentrated and dilute (2 N) hydrochloric acids. Optimal pH of 7−8 is necessary for obtaining good quality product in reasonable yield. At the same time it is important to keep the volume as minimum as possible so that the product precipitates out of solution when the pH is between 7 and 8. This fine balance of optimal pH and volume is especially important for a small-scale synthesis for obtaining satisfactory results.

In a typical procedure, students add 1 mL of conc HCl dropwise to an ice-cold solution of the reaction mixture with constant swirling and monitoring of the pH. After adding 1 mL of concd HCl, they switch to 2 N HCl and continue the neutralization process until a pH of 7−8 is reached. Addition of one extra drop of HCl is usually enough to protonate the free amino group

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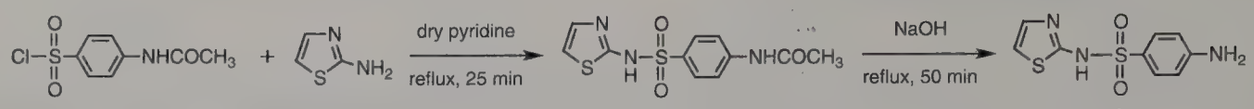
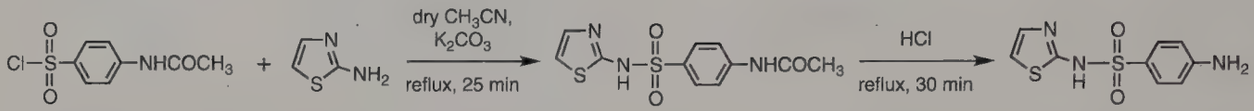
Scheme 1. Synthesis of Sulfathiazole (Old Method)



1 2 3 4

Scheme 2. Synthesis of Sulfathiazole (New Method)



1 2 3 4

of sulfathiazole resulting in very low yield because the protonated form of sulfathiazole is soluble in water. Many students found this neutralization step quite tricky to handle, especially near the end point and, often, after acidification led to a dramatic decrease in the yield and quality of the product sulfathiazole **4**.

■ NEW SYNTHETIC SCHEME

Replacement of Pyridine

To avoid using the toxic chemical pyridine in the large undergraduate teaching laboratory, alternative routes were explored for the synthesis of sulfathiazole that delivered the same learning objectives. Different organic as well as inorganic bases such as triethylamine (TEA), *N*-methyl morpholine (NMM), and anhydrous potassium carbonate were explored as alternatives for pyridine for the synthesis of sulfathiazole. Neither TEA nor NMM generated *p*-acetamidobenzenesulfonamide **3** as a solid product from the condensation of sulfonyl chloride **1** with amino thiazole **2** in dry acetonitrile. Instead, in both cases, a black tar resulted that was not worthy of any further analysis. On the other hand, condensation of sulfonyl chloride **1** with amino thiazole **2** to form the intermediate *p*-acetamidobenzenesulfonamide **3** in dry acetonitrile using anhydrous K_2CO_3 resulted in a light brown solid, which was confirmed to be the desired intermediate **3** based on NMR and MS data. The use of TEA or NMM for this reaction was not explored any further. The reader is encouraged to refer to other literature methods for the synthesis of sulfonamides under different conditions involving the use of various solvents and bases, as well as microwave assisted syntheses.[12−14]

The hydrolysis of the *p*-acetamido group of intermediate **3** by aqueous acid followed by neutralization with solid sodium bicarbonate would simplify the neutralization step and serve as a better option for undergraduate students. Use of solid sodium bicarbonate for the neutralization step eliminates the complications of over acidification as well as over dilution leading to poor yield and lower quality product. This modified route for the synthesis of sulfathiazole is shown in Scheme 2. The only concern was if the heterocyclic ring would be stable to acid hydrolysis conditions. To test this, the laboratory instructors carried out the synthesis of sulfathiazole **4** using the modified procedure shown in Scheme 2. The quality of the sulfathiazole synthesized following the modified protocol was confirmed by ^1H and ^{13}C NMR and MS analysis. The NMR data of the synthetic sulfathiazole obtained by this modified route was found to match perfectly with that of the commercial sample. The synthetic sulfathiazole obtained by the modified route was also tested for antibacterial activity against *E. coli* in comparison to the commercial sample. NMR and MS spectra and results of the antibacterial activity tests are provided as part of the Supporting Information.

Table 1. Results from the Hydrolysis of *p*-Acetamidobenzenesulfonamide to Sulfathiazole

Reflux Time/min	*p*-Acetamidobenzenesulfonamide, 3/g	Sulfathiazole, 4/g	R_f[a]	M + H$^+$
20	0.502	0.174	0.57	256.0195
30	0.506	0.185	0.57	256.0193
40	0.502	0.178	0.57	256.0194
50	0.503	0.206	0.57	256.0191

[a] TLC solvent system was DCM-MeOH (9:1).

Shortening the Hydrolysis Time

To determine if shortening the hydrolysis time would have detrimental effects on product yield or quality, the progress of the acid-catalyzed hydrolysis of the intermediate, *p*-acetamidobenzesulfonamide **3**, to sulfathiazole **4** was monitored as a function of time every 10 min starting from 20 to 50 min. Four parallel reactions were set up using the intermediate **3** synthesized from the same batch. The product isolated at 20, 30, 40, and 50 min, respectively (described in the Supporting Information), recrystallized from hot ethanol, and were characterized by TLC and ESIMS. There was no detrimental effect on the quality or yield of the final product between the four reactions. Product yields, R_f values, and ESIMS results obtained from these four reactions are summarized in Table 1. The 30 min hydrolysis time was chosen, this reaction was repeated several times, and reproducible results were obtained. Thus, a reflux time of 30 min is recommended for the acid-catalyzed hydrolysis of the intermediate *p*-acetamidobenzenesulfonamide **3** to sulfathiazole **4** for undergraduate classes.

■ HAZARDS

4-Acetamidobenzenesulfonyl chloride is corrosive and is harmful by inhalation, in contact with skin, and if swallowed. Acetonitrile is a highly flammable liquid and may be harmful by inhalation, ingestion, or skin absorption. 2-Aminothiazole may cause skin and eye irritation and may be harmful if swallowed. Hydrochloric acid is corrosive and causes burns to all body tissue. Potassium carbonate and sodium bicarbonate can cause eye

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Table 2. Student Yields of Sulfathiazole Using the New Synthetic Route

Year	Student Yield (%)										
2009	41	35	39	48	37	47	42	48	32	28	34
2010	46	50	42	41	45	48	47	44	45	32	44

irritation and ingestion of large amounts may cause diarrhea, nausea, vomiting, and respiratory irritation.

■ STUDENT RESULTS

This modified procedure for the synthesis of sulfathiazole was executed in our medicinal chemistry undergraduate laboratory in 2009 and 2010. A range of percentage yields of sulfathiazole reported by students from the academic years 2009 and 2010 are summarized in Table 2.

■ CONCLUSION

The modified synthetic procedure described here has the advantages of excluding the toxic chemical pyridine from undergraduate laboratories as well as the use of a simplified procedure for the hydrolysis of the *p*-acetamido group of intermediate **3**, reaction work up, and recovery of the final product. Reaction time for the hydrolysis step has also been shortened without any detrimental effect on overall yield or quality of the product. To the best of our knowledge, the syntheses of sulfathiazole and its derivatives reported in the literature as well as followed in most undergraduate teaching laboratories use pyridine as a solvent and base for the condensation of sulfonylchloride with amino thiazole.[4] This is the first report of the synthesis of sulfathiazole that excludes pyridine from the procedure and standardized in a way for convenient execution in large undergraduate teaching laboratories.

■ ASSOCIATED CONTENT

Supporting Information

Laboratory notes for the students; additional notes for the instructors and bioassay results; NMR and MS spectra. Answers to the postlab questions are available from the author. This material is available via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

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Synthesis of Ethyl Nalidixate: A Medicinal Chemistry Experiment

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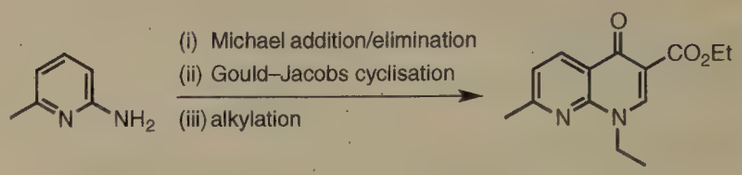
Supporting Information

ABSTRACT: A series of laboratory experiments that complement a medicinal chemistry lecture course in drug design and development have been developed. The synthesis of ethyl nalidixate covers three separate experimental procedures, all of which can be completed in three, standard three-hour lab classes and incorporate aspects of green chemistry such as solvent-free reaction conditions and minimizing purification procedures. The experimental procedures are straightforward and require no specialized equipment, although access to IR and NMR spectrometers for spectral analysis is beneficial. Both the experimental and theoretical aspects of the synthesis are sufficiently challenging for a year-two undergraduate chemistry class.



KEYWORDS: Second-Year Undergraduate, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Drugs/Pharmaceuticals, Green Chemistry, IR Spectroscopy, Medicinal Chemistry, NMR Spectroscopy, Synthesis

A recent restructuring of the undergraduate courses necessitated the introduction of a new year-two medicinal chemistry laboratory. Despite the wealth of literature available for designing synthetic organic chemistry laboratory classes,[1,2] most of the experiments were not wholly applicable to a medicinally oriented class. The syntheses of aspirin,[3] paracetamol,[4] and phenacetin[5] are well documented and some of these have been adopted as experimental exercises to introduce more complex strategies. However, neither the laboratory nor theoretical aspects of these laboratory preparations were sufficiently complex, and a multistep synthesis that was challenging with respect to both experimental and mechanistic organic chemistry was desired.

Several excellent publications have appeared in recent years outlining advances in drug discovery,[6,7] but unfortunately, a majority of synthetic targets were deemed unsuitable for a laboratory teaching class due either to the complexity of synthesis or cost implications. A three- or four-stage preparation was desired where each step was achievable in a 3-h time scale. To this end, the synthesis of nalidixic acid (**1**) appeared to satisfy the criterion (Figure 1).

Although this target is not a widely prescribed drug, its discovery in the early 1960s[8] has led to the development of a range of quinolone antibiotics[9,10] such as ciprofloxacin (**2**) and levofloxacin (**3**). Thus its synthesis complements a lecture course concerned with drug discovery and development.

SYNTHETIC OVERVIEW

The literature synthesis of nalidixic acid is a three-pot, four-step strategy as outlined in Scheme 1. The starting material for the sequence is the commercially available 2-aminopicoline (**4**) that reacts readily with diethyl ethoxymethylenemalonate (**5**) under solvent-free conditions to yield enamine **6**. The second step in the reaction scheme involves heating enamine **6** in diphenyl ether, at reflux, to affect a Gould–Jacobs[11] cyclization to give quinolone **7**.

Literature reports indicate that the second step of the reaction suffers from irreproducibility and the reaction appears to be sensitive to temperature, dilution, and reaction times, thus resulting in either partial or full conversion, or in some cases decomposition. These observations prompted an investigation into the scope and limitations of the Gould–Jacobs reaction published elsewhere.[12] The final stage in the literature synthesis involved N-alkylation followed by ester hydrolysis to give nalidixic acid **1**. Again, there were problems associated with adapting this experiment for teaching purposes, most notably with the long reaction times, and so the entire reaction sequence was investigated to determine if a viable lab-based exercise could be developed.

The results of this investigation beginning with synthesis of enamine **6** are outlined in Scheme 2. This experiment follows the literature procedure closely and the experiment is straightforward, high yielding, and may be carried out on the bench in a conical flask that can subsequently be used for the recrystallization procedure. In this case, the temperature can be monitored using a thermometer as the reaction proceeds over a broad temperature range of 100–120 °C. The next stage in the synthesis was more difficult to optimize, but the solvent, dilution, and temperature effects were investigated, and after some considerable effort, a small-scale reaction was developed that was reproducible and required only minimal purification. Thus, performing the reaction in the more thermally stable Dowtherm A (3 g in 30 mL of solvent) at 270 °C gave quinolone **7**, which only required washing with diethyl ether to effect purification.

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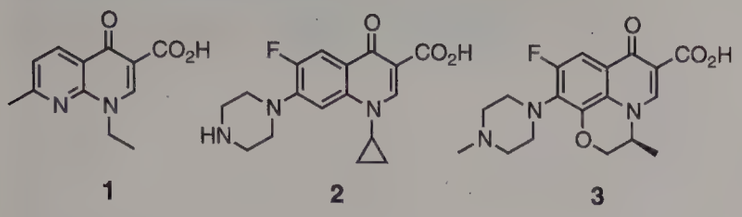
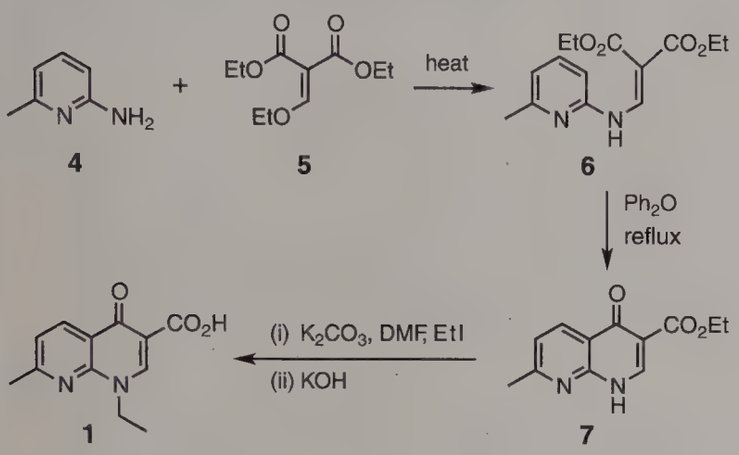
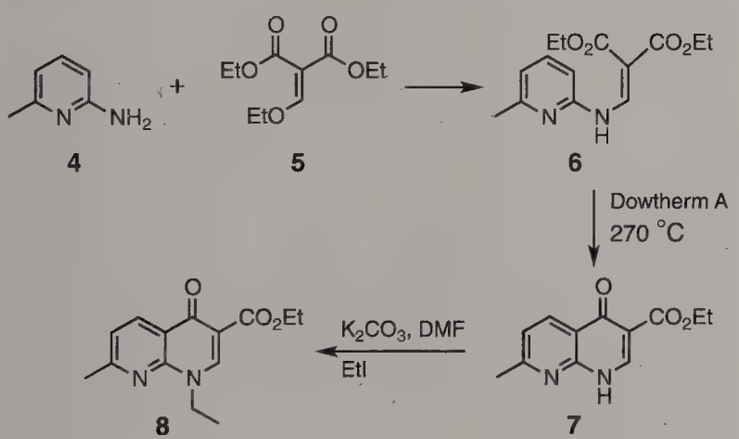


Figure 1. Structures of nalidixic acid (**1**), ciprofloxacin (**2**), and levofloxacin (**3**).

Scheme 1. Literature Synthesis of Nalidixic Acid



Scheme 2. Synthesis of Ethyl Nalidixate



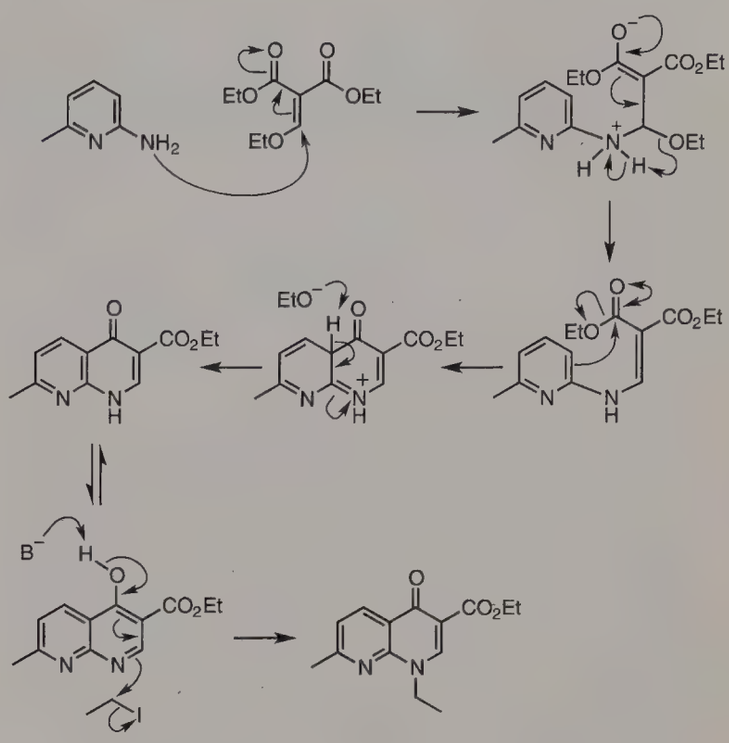
The best way to achieve reaction temperatures of this magnitude was to use an aluminum heating block with a contact thermometer as control to maintain a uniform temperature.

The final stage in the literature synthesis involved N-alkylation of **7** followed by ester hydrolysis to give nalidixic acid, **1**. A two-stage strategy was considered to convert **7** to **1**, but instead, the hydrolysis step was not performed and ethyl nalidixate, **8**, was synthesized. This has the added advantage that a simple workup procedure furnished sufficiently pure material to produce a clean ^{1}H NMR for analysis.

■ METHODS AND MATERIALS

Experiments were carried out using a conventional hot-plate stirrer. IR spectra were recorded on a Perkin-Elmer FT-100 spectrometer and NMR spectra were measured on a JEOL ECX 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). Coupling constants (J) are given in hertz (Hz). All the reagents were obtained from Sigma-Aldrich and used without purification.

Scheme 3. Mechanistic Overview of the Synthesis of Ethyl Nalidixate



■ HAZARDS

Students are advised to carry out all reactions in a fume hood using appropriate protective clothing and eyewear. 2-Aminopicoline is toxic and causes irritation to eyes, skin, and respiratory tract. Diethyl ethoxymethylenemalonate and Dowtherm A cause eye, skin, and respiratory tract irritation. Ethanol and diethyl ether are highly flammable and the latter is prone to peroxide formation. Ethyl iodide and all deuterated solvents should be regarded as possible carcinogens.

■ MECHANISTIC OVERVIEW

The synthesis of ethyl nalidixate proceeds initially via a conjugate (Michael) addition–elimination reaction as outlined in Scheme 3. The second stage in the synthesis displays an example of electrophilic aromatic substitution, specifically the Gould–Jacobs reaction, to afford the cyclized product, whereas the final step in the synthesis involves a S_N2 mechanism. Although the latter is more in line with year-one mechanistic organic chemistry, the reaction deals with bifunctional nucleophiles and therefore introduces the more advanced subject of "hard" and "soft" nucleophilic agents.

■ RESULTS AND DISCUSSION

Ethyl nalidixate, the parent compound of a range of quinolone antibiotics, can be prepared in the laboratory in three steps. The synthesis is straightforward, high yielding, and highlights aspects of green chemistry[13] such as minimizing purification and solvent-free reaction conditions. The only concern in the procedure is in the final stage where reaction times of 3 h are required. This is

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relatively easy to deal with as the reaction can simply be switched off at the end of the day (by the lab technician) and is ready for workup during the next lab session.

This lab class experiment has a number of advantages over conventional synthetic organic chemistry experiments. The experiment involves a multistage synthesis, which gives the students an appreciation of the problems associated with target-molecule synthesis. The students are expected to obtain and interpret spectral data (IR and ^{1}H NMR) for the three compounds made in the laboratory and it is worth noting that the ^{1}H NMR for all three compounds are of a level that is sufficiently challenging for a good year-two chemist. The mechanistic chemistry associated with the reactions also covers a range of year-two topics that are incorporated into an assignment in addition to the laboratory and writeup.

In the initial trials for this laboratory, the students spent a lot of time waiting for the reactions to complete their run-time. Therefore, a second, two-stage synthesis was introduced to run alongside the nalidixic acid synthesis that involved preparing paracetamol, and subsequently phenacetin, from aminophenol. Student feedback from this course has now proved extremely positive with the students commenting on their increased appreciation of medicinal-chemistry research and their enjoyment of meeting the challenges of "juggling" two separate projects with the resultant development of their lab-skills.

■ ASSOCIATED CONTENT

Supporting Information

The experimental conditions for the reactions and physical data such as melting points and spectroscopic data; the laboratory manual for students, which includes the synthesis of paracetamol and phenacetin as well as a brief background on quinolone antibiotics; CAS numbers for all chemicals used in the laboratories. This material is available via the Internet at http://pubs.acs.org.

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Synthesis of Two Local Anesthetics from Toluene: An Organic Multistep Synthesis in a Project-Oriented Laboratory Course

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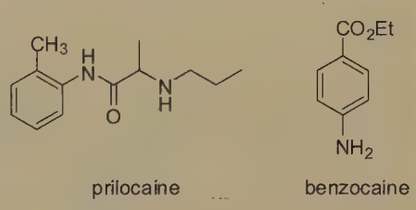
Supporting Information

ABSTRACT: This article describes one of the projects in the advanced undergraduate organic chemistry laboratory course concerning the synthesis of two local anesthetic drugs, prilocaine and benzocaine, with a common three-step sequence starting from toluene. Students undertake, in a several-week independent project, the multistep synthesis of a pharmaceutical drug, comprising instructor-guided tasks such as literature search, planning, critical discussion, experimental design, observation, and results interpretation. In this project, in addition to searching and using information found in primary and secondary sources, students learn to design the methodology for several of the steps in the reaction sequence, bearing in mind safety and environmental concerns.



KEYWORDS: Upper-Division Undergraduate, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Problem Solving/Decision Making, Amines/Ammonium Compounds, Chromatography, Drugs/Pharmaceuticals, NMR Spectroscopy, Synthesis

Our academic approach is based on the premise that learning is facilitated in a research environment, which involves independent study, decision making, and problem solving through research projects.[1−3] To this end, a nontraditional advanced undergraduate organic chemistry laboratory course was developed. The project-oriented system consists of half-semester research projects (about eight 4-h lab sessions) that involve the multistep synthesis of a variety of classical pharmaceutical drugs, with methodologies usually adapted from primary literature. One of these projects is described; the multistep synthesis of two local anesthetic drugs, benzocaine and prilocaine, from a common three-step sequence starting from toluene that requires the student to formulate a hypothesis and to design experiments to test it.

LABORATORY DYNAMICS

Students work singly or in pairs, with different projects. Each instructor is in charge of up to 10 students. The semester work starts with three to five simple experiments, presented in a nonconventional laboratory manual (more accurately, a laboratory guide) that includes: (i) an introduction describing the work system; (ii) 40 experimental proposals (each featuring a reaction scheme, main objective, procedure references, a study guide, prelab questions, and notes, as well as IR and NMR spectra); and (iii) 10 appendixes addressing topics such as the lab notebook, experimental design, microscale, waste disposal, bibliographic research, and laboratory rules (the original version of this manual, in Spanish, is included in the Supporting Information).

Before conducting each experiment, students search for information, discuss it with the instructor, and outline a laboratory work plan, generating a grade that will be averaged with the lab work and report. In this preliminary evaluation, the instructor inquires about the theoretical background of the experiment (reaction type, mechanism, stoichiometry, etc.) and the procedure, frequently advising students against the idea that they must "follow instructions step-by-step" to achieve success. This interaction is intended to promote a critical process that allows students to design their own work plan and gives them self-confidence. The dialogue between the students and instructor may be time-consuming, but the instructors must be aware of the work the students are carrying out, making sure that the students know, before initiating each step, how they are going to do it, why, and what to expect.

At the beginning of the course, pharmaceuticals are assigned to the students, who do a time-constrained bibliographic search in Chemical Abstracts (CA), either in printed form or through the SciFinder database, seeking information on syntheses for the assigned drug. This search (typically 1950 through 1980) frequently renders several nonrecoverable papers or patents, so it is sometimes necessary to discuss alternatives, and then plan the project and design experimental work based on nondetailed procedures outlined in CA, turning the process into authentic laboratory research. Laboratory limitations (time, materials, and equipment) are taken into account in choosing a synthetic strategy, as well as in designing each experiment; nonetheless, additional support from a research laboratory is sometimes necessary for reagents and facilities, which facilitates the teaching−research link. Students learn that, even for the reproduction of methodology from a formal paper, adaptations or modifications may be necessary, so they are encouraged to initially explore

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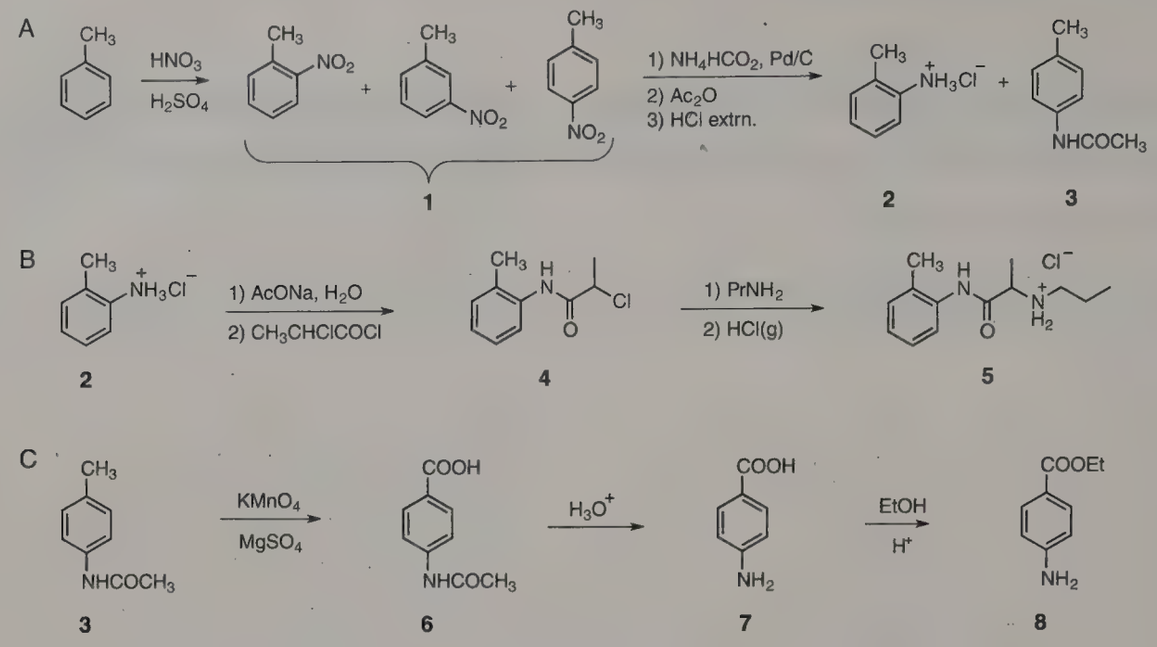
Scheme 1. Divergent Synthesis of Prilocaine Hydrochloride (5) and Benzocaine (8)



uncertain procedures on a microscale. From a rough calculation of the expected global yield, the quantity of starting material is calculated to obtain about 1 g of final product. Students typically encounter chemical transformations that have not been covered in lecture, highlighting the importance of qualified laboratory instructors, as well as good communication with lecture instructors.

More than the successful culmination of the synthesis, the learning process and the interest the students demonstrate in understanding their experiments are valued. For each experiment, a grade is assigned on the basis of (i) preliminary oral evaluation (correlation between theory and practice, planning of the experiment), (ii) performance in the lab (attention to lab guidelines, time management, responsibility, ethics, etc.), and (iii) proper documentation in a lab notebook. A final-term report is assigned on the synthesis and other features (bibliographic research, pharmacology, analysis) of the target drug to encourage the students to have a comprehensive view of the prepared compound. Oral reports to the class are given by the students who carried out the most interesting or successful projects.

■ PROJECT DESCRIPTION

The multistep divergent synthesis of two local anesthetics, prilocaine hydrochloride (5) and benzocaine (8), with a common initial three-step reaction sequence starting from toluene is described as an example project (Scheme 1). Local anesthetics, which reversibly block nerve impulses, can be divided into two main groups, esters (e.g., cocaine, benzocaine, procaine, tetracaine) and amides (e.g., lidocaine, prilocaine, bupivacaine), by the functional group that connects the hydrophobic group (generally an aromatic ring) and the hydrophilic group (frequently a secondary or tertiary amine group).[4] Side effects of local anesthetics are common; a metabolite of benzocaine is *p*-amino benzoic acid, which is associated with allergic reactions, and a breakdown product of prilocaine, *o*-toluidine, can produce methemoglobinemia. Also, prilocaine has been reported to induce apoptosis in osteoblastic cells.[5] Prilocaine is used as a racemate, although isomers differ in potency and in toxicity.[6]

This project, undertaken by two students as a team, illustrates several classic reaction types and involves many common laboratory techniques. Most importantly, it requires students to design a methodology for the conversion of the nitrotoluene isomer mixture obtained in the first step (part A, Scheme 1) into the starting material for each local anesthetic. The methodology is based on a hypothesis, which can easily be verified, concerning the different reactivity of the corresponding amines.

Nitration of toluene is a classical example of an electrophilic aromatic substitution on an activated benzene ring, which affords a mixture of nitrotoluene isomers in varying ratios depending on the specific conditions. In this project, students have employed the classical sulfuric/nitric method by adapting a laboratory procedure published in this *Journal*,[7] leading to a mixture of *o*-, *m*-, and *p*-nitrotoluene (MNT) isomers (1) in a typical ratio of about 59:4:36, respectively, with yields of 62−80%. An alternative nitration method, which students have reproduced, employs in situ generated acetyl nitrate.[8] Because the separation of the MNT isomers is too difficult to accomplish in the laboratory,[9] the isomeric ratio is established by GC or HPLC analysis and the mixture is used as the starting material for the synthesis of target products.

Reduction of the MNT mixture by catalytic hydrogen transfer (ammonium formate, Pd/C, AcOEt)[10] affords a mixture of toluidine isomers, from which the *p*-toluidine may be selectively acetylated, owing to its reduced steric demand. After discussing the steric properties of the toluidines with the instructor, the students in charge of the project must propose a hypothesis regarding which of the isomers will be the most reactive toward an acylating agent, develop a work plan to verify it, and a strategy that allows them to isolate both main products, *o*-toluidine and *p*-methylacetanilide. To achieve this, students treat the dried solution of the toluidine isomer mixture, at 0 °C, with one molar equivalent (as to the amount of *p*-isomer theoretically present in the mixture) of acetic anhydride. TLC analysis of the reaction mixture allows a clear differentiation of all compounds involved (see the Supporting Information). Following dilute HCl extraction, *p*-methylacetanilide (3) is isolated and purified by one of the students as the starting material for benzocaine synthesis.

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The *o*-toluidine hydrochloride solution (**2**) is used by the other student for prilocaine hydrochloride synthesis.

The synthesis of prilocaine hydrochloride (**5**) from **2** (part B in Scheme 1) is accomplished through modifications of the described methodologies[11] to suit the laboratory conditions and small scale. The *o*-toluidine hydrochloride solution (**2**) is buffered to pH = 5−6 (NaOH and AcONa) and cooled to 5 °C before adding 2-chloropropionyl chloride to afford chloroamide **4** (mp 112−113 °C). Overall yields of **4** from toluene (no isolated intermediates) are typically in the range from 12 to 26%. Synthesis of prilocaine hydrochloride (**5**) was simplified by allowing a solution of **4** in *n*-propylamine to stand for two days at room temperature, followed by treatment of the isolated base product with gaseous HCl, either lab generated[12] or by the use of a commercially available hydrogen chloride/2-propanol solution. Typical yields of **5** from **4** are around 60−80 %.

By omitting several isolation and purification steps, this set of operations may be considered a telescoping synthesis. This is a green chemistry strategy, where one reactant goes through multiple transformations without isolation of intermediates, and is aimed to reduce the number of unit operations, in this way saving time, reducing environmental burden (solvents, energy, etc.), reducing the need to manipulate toxic materials, and increasing yield.[13]

Benzocaine (**8**) is prepared from *p*-methylacetanilide (**3**) (part C in Scheme 1) in a three-step reaction sequence, involving procedures described in this *Journal*[14] and in several laboratory instruction manuals (overall yields of about 12 to 22 %).

■ HAZARDS

All reactants, products, and solvents must be handled in a manner consistent with the information available on their Material Safety Data Sheets (MSDS). Eye protection and gloves must be worn at all times and procedures must be conducted in a fume hood. Nitrotoluene and toluidine isomers are skin irritants and suspected carcinogens. Acetic anhydride is a lachrymator, corrosive, and flammable. 2-Chloropropionyl chloride should be handled with particular care as it is extremely corrosive, lachrymator, water-reactive (generating HCl), and flammable. Toluene, methanol, diethyl ether, hexane, ethyl acetate, and isopropyl alcohol are all volatile, toxic, and flammable liquids; particularly, diethyl ether should be kept away from sparks or fire. Propylamine is highly volatile, toxic, flammable, and irritating to the skin and mucous membranes. Ammonium formate and sodium acetate trihydrate are irritant to skin and eyes. Sodium hydroxide is very caustic. Sulfuric, nitric, and hydrochloric acids are corrosive to eyes, skin, and mucous membranes. Palladium on carbon is pyrophoric when dry; it can cause fire in contact with combustible materials, such as organic solvents or filter paper. Hydrogen chloride is extremely corrosive and irritating to eyes, skin, and mucous membranes.

■ CONCLUSIONS

This project was developed and refined with the work of students from several generations in our course. It requires the design of a telescoping sequence for several of the synthetic steps, as well as adaptations to previously reported syntheses, to suit laboratory conditions. Students have described this project as challenging and useful, as it has allowed them to practice a variety of experimental techniques and reaction types, in addition to exposing them to real scientific practice.

■ ASSOCIATED CONTENT

Supporting Information

Background information, experimental procedure with notes for instructors, safety hazards, list of chemicals, gas and HPLC chromatograms, NMR spectra. The lab manual (in Spanish) used in our course is included in a separate compressed file. This material is available via the Internet at http://pubs.acs.org.

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Development of an Interdisciplinary Experimental Series for the Laboratory Courses of Cell and Molecular Biology and Advance Inorganic Chemistry

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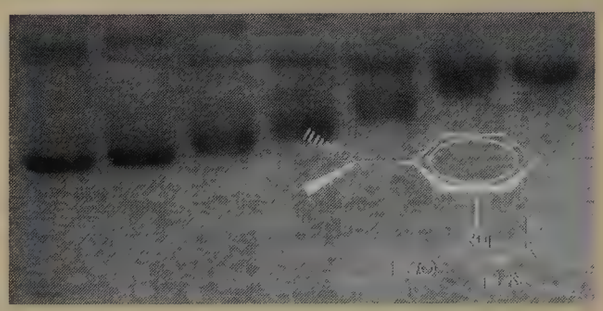
Supporting Information

ABSTRACT: An interdisciplinary approach to education has become more important in the development of science and technology, which requires universities to have graduates with broad knowledge and skills and to apply these skills in solving real-world problems. An interdisciplinary experimental series has been developed for the laboratories in cell and molecular biology and advanced inorganic chemistry. Students performed the synthesis and characterization of a Ru(II) monoarene anticancer complex in the advanced inorganic chemistry laboratory, followed by testing its DNA-binding properties in the cell and molecular biology laboratory. The objectives of this experimental series are to introduce the students to the connections between different scientific subjects and to the importance of an interdisciplinary approach in solving practical scientific problems. Students learn important techniques such as synthesis and purification, ^{1}H NMR and IR spectroscopy, and electrophoresis that are used collectively in the development of pharmaceuticals.



KEYWORDS: Upper-Division Undergraduate, Biochemistry, Inorganic Chemistry, Interdisciplinary/Multidisciplinary, Laboratory Instruction, Collaborative/Cooperative Learning, Hands-On Learning/Manipulatives, Coordination Compounds, Drugs/Pharmaceuticals, Molecular Biology

With the rapid advancement of science and technology in recent years, the boundaries between traditional scientific disciplines have become increasingly blurred. Scientists from different disciplines are routinely working together to solve real-world problems. To produce graduates who can adapt to the interdisciplinary working environment, colleges and universities are reforming their curricula to meet the needs of students. Many interdisciplinary degree programs have been generated such as chemical biology or chemical physics, and so forth; the curricula of traditional degree programs have become more and more diverse and flexible to allow students to take classes from disciplines other than their majors; faculty are working to incorporate more information about the real-world applications of the subject matter into their courses. However, the credit hours in a semester and in a degree program are limited. Educators are often faced with the dilemma of how to incorporate more information related to the application of the subject matter into the course without cutting back too much on the fundamental materials covered.

One solution to this dilemma is to establish closer collaborations between faculty members of different disciplines who are trying to teach interdisciplinary content. One format of this type of collaboration is to develop a comprehensive project around a common theme that involves two or more subjects or disciplines. The faculty member of each subject incorporates a portion of the project in his or her class. Students complete a part of the project after taking each class and complete the whole project after taking all the courses involved. Through this type of project, students learn how knowledge and skills of different subjects or disciplines can be used collaboratively to solve a common real-world problem. Each faculty member is able to focus on in-depth coverage of the portion of the project that is within his or her expertise instead of spending time providing superficial coverage on the applications of the subject in other areas outside their expertise. This format not only saves class time, but also provides a more comprehensive learning experience for the students. Many projects of this type have been developed between various subjects of chemistry[1] and between chemistry and other disciplines.[2,3] For example, an integrated laboratory sequences that provides students with research-like experiences in organic synthesis and spectroscopic analysis has been developed by Ball and Miller;[1] Woo and coworkers have described interdisciplinary projects developed through a collaborative effort of chemistry and computer science faculty.[2] Educational projects that relate instrumental analysis with art restoration have also been reported.[3]

An interdisciplinary experimental series has been developed for the laboratories in cell and molecular biology and advanced inorganic chemistry. Students performed the synthesis and characterization of a Ru(II) monoarene anticancer complex in the advanced inorganic chemistry laboratory, followed by testing

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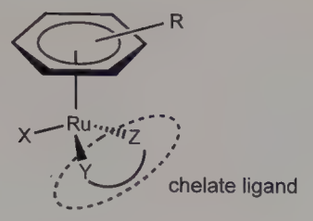

Figure 1. Generalized structure of potential anticancer Ru(II) monoarene complexes.

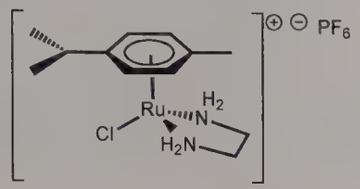

Figure 2. Structure of $[(\eta^6\text{-p-cymene})RuCl(H_2NCH_2CH_2NH_2\text{-}N,N')]PF_6$.

its DNA-binding properties in the cell and molecular biology laboratory.

Platinum complexes are widely used in cancer treatment but they have some serious problems. For example, complexes of platinum induce drug resistance, toxic side effects, and cytotoxicity toward limited types of cancer.[4] Therefore, searching for agents of other transition-metal complexes is currently an active research area.[5]

Ruthenium complexes have been extensively studied as potential anticancer agents.[5,6] Two Ru(III) complexes are currently undergoing clinical trials: *trans*-$[RuCl_4(DNSO)Im]ImH$[7] (NAMI-A, Im = imidazole)[8] and *trans*-$[RuCl_4(Ind)_2]IndH$ (KP1019, Ind = indazole).[9] Studies have shown that the activity of Ru(III) complexes is dependent on its in vivo reduction to more labile Ru(II) complexes.[10] Therefore, anticancer activities of Ru(II) monoarene complexes with a general structure shown in Figure 1 have also been explored.[7]

Similar to platinum complexes, Ru(II) monoarene complexes can also bind DNA selectively at guanine bases.[7] However, studies have shown that these compounds are also active against cisplatin-resistant strains of ovarian cancer cells,[11] which indicates that the mode of action is different from that of platinum compounds. In addition, these complexes are less reactive than cisplatin toward proteins and other biological compounds in the blood stream, which may lead to a cancer treatment with fewer side effects.[7] Finally, these Ru(II) compounds have good water solubility and most of them are easy to synthesize.[12]

As a result, one of the Ru(II) monoarene complexes, $[(\eta^6\text{-}p\text{-cymene})RuCl(H_2NCH_2CH_2NH_2\text{—}N,N')]PF_6$ (Figure 2) was used as the target molecule for the interdisciplinary experimental series. In the advanced inorganic laboratory, students performed the synthesis and spectroscopic characterization of the complex. The students then explored the DNA-binding properties of the compound in the cell and molecular biology laboratory.

■ PEDAGOGY

The pedagogy for this interdisciplinary laboratory emphasizes how knowledge and techniques in two different subject areas can be used to solve real-world problems. The students learn the biomedical applications of inorganic chemistry and molecular biology, specifically in the development of new anticancer drugs, thus, enhancing their interest in learning both subjects.

■ ADVANCED INORGANIC CHEMISTRY LABORATORY

The inorganic chemistry laboratory is a one-credit chemistry laboratory with a total meeting time of 30 h per semester (3 h/wk). The synthesis of the anticancer agent $[(\eta^6\text{-}p\text{-cymene})RuCl(NH_2CH_2CH_2NH_2\text{—}N,N')]PF_6$ used two laboratory periods in the advanced inorganic chemistry laboratory. The students synthesized the complex in week one and carried out spectroscopic characterization in the second week.

Synthesis of $[(\eta^6\text{-}p\text{-Cymene})RuCl(H_2NCH_2CH_2NH_2\text{—}N,N')]PF_6$

The Ru(II)—cymene complex was synthesized according to the published method (Scheme 1).[12] Both $[(\eta^6\text{-}p\text{-cymene})_2RuCl_2]_2$ and ethylenediamine were purchased from Sigma-Aldrich and used without further purification. The reaction was performed in a fume hood. In a 150 mL three-neck round-bottom flask, 0.19 g (0.32 mmol) of $[(\eta^6\text{-}p\text{-cymene})RuCl_2]_2$ and 30 mL of dry methanol were combined and degassed. Ethylenediamine, 0.06 g (1 mmol), was added in one portion using a microsyringe. The reaction was stirred at room temperature for 60 min under nitrogen flow and covered with aluminum foil. Then, the reaction mixture was filtered using a glass filter and NH_4PF_6, 0.26 g (1.6 mmol), was added. The solution volume was reduced to about 7—8 mL on a rotary evaporator. The filtrate was cooled in an explosion-proof refrigerator overnight. The product was collected by filtration and washed with a small volume of ether.

Spectroscopic Characterization

The ^1H NMR spectrum of the complex was taken in deuterated DMSO with a Varian 400 MHz NMR spectrophotometer. The infrared spectrum was taken using a KBr pellet with a Nicolet FT-IR spectrophotometer.

Hydrolysis of $[(\eta^6\text{-}p\text{-Cymene})RuCl(H_2NCH_2CH_2NH_2\text{—}N,N')]PF_6$

The stock solution of 0.1 mM $[(\eta^6\text{-}p\text{-cymene})RuOH_2(H_2NCH_2CH_2NH_2\text{—}N,N')]PF_6$ was prepared in water and it was protected from light and left at room temperature for at least 24 h for the hydrolysis to occur.

■ CELL AND MOLECULAR BIOLOGY LABORATORY

Molecular and cell biology is a two-credit laboratory course with a total meeting time of 40 h per semester (4 h/wk). The series of experiments used two laboratory periods: one to isolate the DNA and the other to test the biological properties of the compound.

Plasmid Preparation

The plasmid pETBlue2-CRABPII (3900 bp) was isolated from XL1-Blue cells using Qiagen's Mini Prep DNA isolation kit. The suggested procedure was used without modifications. The DNA was quantified using the A_{260} and the plasmid was frozen until used.

Incubation of Plasmid with $[(\eta^6\text{-}p\text{-Cymene})RuOH_2(H_2NCH_2CH_2NH_2\text{—}N,N')]PF_6$

A series of samples with different Ru—cymene/base pairs (r_b) ratios were prepared using the hydrolyzed Ru—cymene complex. The samples were incubated for 48 h in the dark at 37 °C.

Electrophoresis

The samples were loaded on a 1% agarose gel and run at 30 V for 2.5 h. Fast Blast DNA stain was used as a stain for DNA

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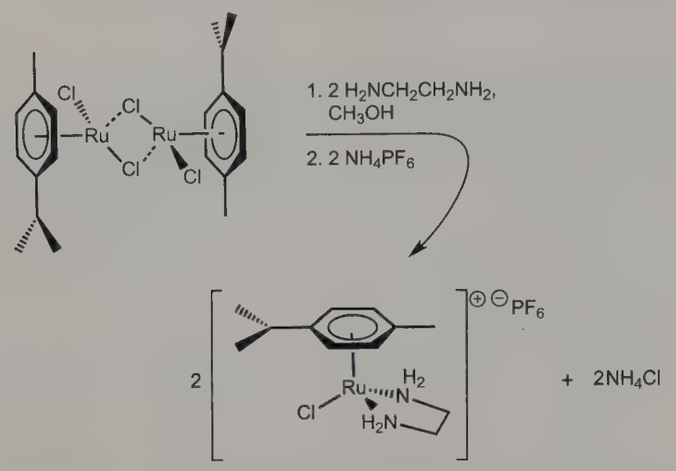
Scheme 1. Synthesis of Ru(II) Monoarene Complex



(ethidium bromide is not recommended due to interference with DNA).

■ HAZARDS

All students must wear safety eye protection and gloves. They must follow the general safety precautions required in chemistry laboratories. The students should examine the MSDS sheets for the specific hazards associated with the chemicals used in the experiment. The reaction must be performed in a fume hood. All waste must be collected and disposed of according to safety regulations.

■ RESULTS AND DISCUSSION

Complex Synthesis

The Ru(II) monoarene complex, $[(\eta^6\text{-}p\text{-cymene})RuCl(H_2NCH_2CH_2NH_2{-}N,N')]PF_6$, was synthesized by the reaction between $[(\eta^6\text{-}p\text{-cymene})_2Ru_2Cl_2]_2$ and ethylenediamine. The reaction for the synthesis is shown in Scheme 1. This is an important reaction for Ru(II) chemistry because it is a common reaction to prepare Ru(II) monoarene complexes.[13] By varying the arene ligand of the Ru(II) dimer and the nature of the incoming ligand, various Ru(II) monoarene complexes can be produced. This reaction has been used to prepare a series of different Ru(II) monoarene complexes for structural–activity studies.[12,14]

The synthesis was performed using anhydrous methanol and under nitrogen to prevent hydrolysis. It was also protected from light by covering the flask with aluminum foil. The raw product was filtered to remove any possible insoluble byproduct. Then, NH_4PF_6 was added and the volume of the solvent was reduced to about 7–8 mL. It took about several hours or overnight at 4 °C for the product to crystallize. As a result, large pure crystals could be obtained. The synthesis could be completed in a 3-h laboratory period and the product could be kept at 4 °C for a week until the next laboratory period. However, depending on the number of rotary evaporators available in the laboratory, sometimes students had to come back to finish the rotary evaporation step. In addition, if no crystals form after being kept at 4 °C overnight, the volume of the reaction mixture should be reduced further by rotary evaporation. In that case, students might have to come to the laboratory outside class time to carry out the rotary evaporation step. The product was stable in air at room temperature.

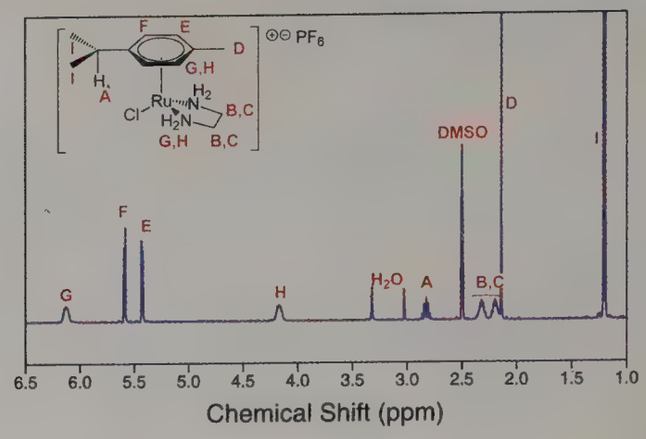


Figure 3. Proton NMR spectrum of $[(\eta^6\text{-}p\text{-cymene})RuCl(H_2NCH_2CH_2NH_2{-}N,N')]PF_6$.

However, for long-term storage, it should be stored in an amber bottle to prevent possible photodecomposition.

The reaction was easy to perform with good % yield (50–70%). The amounts of starting materials used were about half of what was reported in the literature[12] without the reduction of yield. Students are introduced to an important organometallic reaction as well as have an opportunity to practice common laboratory techniques such as inert atmosphere synthesis, product isolation, filtration, and crystallization. The product is a "half-sandwich" organometallic complex, which is an important topic in the lecture class. The nature of the bond between the arene and metal center contributes to the stability of Ru(II) by d(π)-p(π) back bonding.

Spectral Characterization of the Complex

Because of its ionic nature, the product, $[(\eta^6\text{-}p\text{-cymene})RuCl(H_2NCH_2CH_2NH_2{-}N,N')]PF_6$, is soluble in water and DMSO. ^1H NMR spectra of the product dissolved in DMSO were recorded using 400 MHz NMR. D_2O was not used as a solvent because the complex would hydrolyze in D_2O with the replacement of chlorine by D_2O. However, ^1H NMR could be used to study the hydrolysis reaction of the product, which could be an extension of the experiment. Clean spectra were obtained, which indicated that the product was pure. Because there are several types of protons present in the molecule including protons that are bound to aliphatic carbon, nitrogen, and the aromatic ring that is coordinated to the metal, the NMR spectrum is a good example for teaching NMR of organometallic compounds.

The most interesting features of the ^1H NMR spectrum (Figure 3) are the unique chemical shifts of the aromatic proton peaks E and F and the splitting pattern of the methylene proton peaks B and C on the ethylenediamine ligand. Both features indicate that the ligands are coordinated to the metal. The chemical shift of the aromatic protons on a free cymene should be around 7.0 ppm. However, the chemical shift of the aromatic proton in the Ru(II) product occurs at around 5.5 ppm, which indicates that the deshielding effect of the aromatic ring is reduced by coordinating to Ru(II). The four methylene protons in a free ethylenediamine molecule should give one singlet. However, there are two multiplets (B and C) in the spectrum of the Ru(II) complex. This can be explained because ethylenediamine is coordinated to the metal as a chelate ligand in a nonsymmetrical environment. Therefore, rotation around the C–C bond is no longer allowed, which leads to two different protons on each methylene carbon, one pointing toward the cymene ring and the

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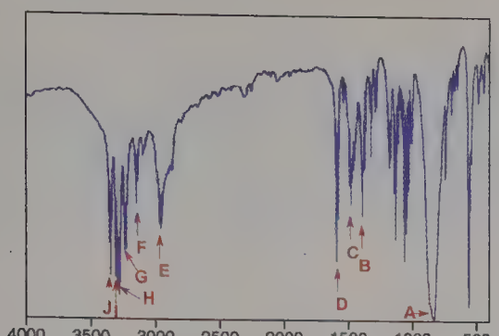


	Position			Position	
A	830.54	Bz para	F	3148.41	CH-sp^2
B	1389.27	C-N	G	3235.23	NH_{ABCD}
C	1478.52	CH_3	H	3284.03	
D	1586.90	Bz para	I	3304.70	
E	2962.12	CH-sp^3	J	3350.58	

Figure 4. FTIR spectrum of $[(\eta^6\text{-}p\text{-cymene})RuCl(H_2NCH_2CH_2NH_2\text{-}N,N')]PF_6$.

other pointing down away from the cymene ring. The amine protons (G, H) show different chemical shift due to the formation of the complex with Ru(II).

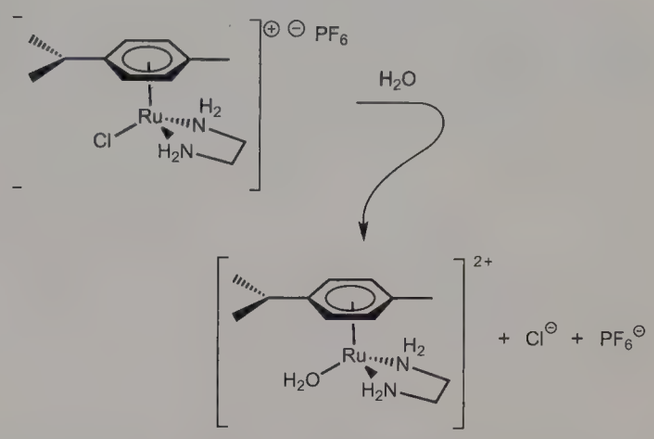
The infrared spectrum (Figure 4) of the product shows the characteristic peaks of the ligands including aromatic C–H, N–H, and aromatic C–C as well as aliphatic C–H bands. If a far-IR instrument is available, metal–ligand bands can also be studied.

DNA Binding Study

Ruthenium(II) monoarene complexes are able to bind DNA, which is believed to be the mechanism of their anticancer activities.[15] It has been reported that the binding of these complexes causes unwinding of the DNA double helix.[16] This is advantageous because DNA unwinding can be easily studied by agarose gel electrophoresis using a circular plasmid DNA. It is well-known that the degree of helical winding and overall DNA topology are intimately related.[17] Plasmids, small circular DNAs that are supercoiled, provide a relatively simple system for studying the effects of the binding of unwinding agents such as anticancer agents. In a circular DNA, such as a plasmid DNA, the sum of the number of duplex turns (α) and the number of super coils (β) is a constant as long as the DNA strands are intact. Because the DNA double helix is right handed and the super helix is left handed, α and β have opposite signs. The more highly supercoiled the circular DNA molecule is, the more compact its structure and the faster it migrates through the agarose gel. Therefore, a compound that unwinds DNA double helix reduces the number of supercoils in a plasmid DNA, which in turn causes a decrease in the mobility of the plasmid DNA.[18]

The plasmid used was the pETBlue-CRABPII, 3900 base pairs (bp); this was chosen due to its easy accessibility. The first step was to prepare and purify the plasmid DNA. Initially, the plasmid is located in *Escherichia coli* XL1-Blue cells. The *E. coli* cells were inoculated and grown overnight prior to the plasmid purification. The incubation could be perform at the end of the previous laboratory and then frozen until use. The plasmid was isolated using Qiagen's Mini Prep DNA isolation kit.[19] The suggested procedure provided by Qiagen was used without modifications. The plasmid DNA purification requires one laboratory period. The DNA was quantified using the A_{260} UV–vis band; the concentration obtained varied from 70 to 170 ng/μL.

Scheme 2. Hydrolysis of $[(\eta^6\text{-}p\text{-Cymene})RuCl(H_2NCH_2CH_2NH_2\text{-}N,N)]PF_6$



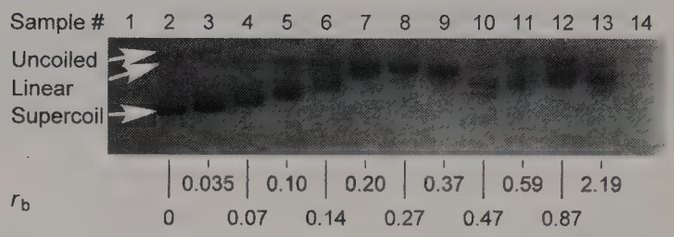


Figure 5. Different degree of DNA coiling at different complex/base pair ratios (r_b) using Fast Blast Blue Stain.

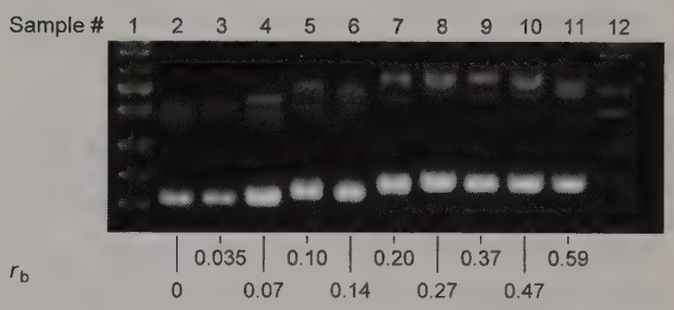


Figure 6. Different degree of DNA coiling at different complex/base pair ratios (r_b) using ethidium bromide.

After the Ru(II) complex was hydrolyzed (Scheme 2) for 48 h, a series of solutions were prepared. The solutions of the hydrolyzed Ru(II) complex were frozen until ready to be used. In the next class period, the Ru complex solutions were combined with the plasmid DNA. To each tube about 1.5 μg of plasmid DNA (3900 bp, assuming avg M_r 649 per bp) and different complex/bp ratios (0, 0.035, 0.07, 0.10, 0.14, 0.20, 0.27, 0.37, 0.47, 0.59, 0.87, and 2.19) were added. The solutions were incubated in the dark at 37 °C for 48 h. The preparation of these solutions requires about 30 min. Because of the short time used, it was chosen to perform this during class time (2 days before the laboratory).

After the incubation, the uncoiling of DNA was examined using agarose (1%) electrophoresis. It was important that the voltage was set at 30 V for optimal resolution of different DNA coiling stages. The completion of the electrophoresis gel required about 1 h; therefore, this time was used to cover the class material. When the electrophoresis was completed, the DNA was visualized using Fast Blast Blue Stain from Invitrogen (Figure 5). It

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Table 1. Mean Scores for Students Who Took the Inorganic Chemistry Laboratory

	One Laboratory			Two Laboratories		
	Pre-Lab[a]	Post-Lab[a]		Pre-Lab[a]	Post-Lab[a]	
Statement	Mean (%)	Mean (%)	Gain (%)	Mean (%)	Mean (%)	Gain (%)
I am able to describe the structure of [Ru(II)(η6-*p*-cymene)(en)Cl]PF_6.	38	75	37	69	88	19
I am able to describe the reaction that produces [Ru(II)(η6-cymene)(en)Cl]PF_6.	38	88	50	69	81	12
I am able to perform inorganic syntheses in an inert atmosphere.	63	88	25	75	100	25
I am able to perform common laboratory procedures used in the experiment such as filtration and rotary evaporation.	88	88	0	75	88	13
I am able to interpret the ^{1}H NMR spectrum of [Ru(II)(η6-cymene)(en)Cl]PF_6.	50	75	25	50	88	38

[a] Agreement.

Table 2. Mean Scores for Students Who Took the Cell and Molecular Biology Laboratory

	One Laboratory			Two Laboratories		
	Pre-Lab[a]	Post-Lab[a]		Pre-Lab[a]	Post-Lab[a]	
Statements	Mean (%)	Mean (%)	Gain (%)	Mean (%)	Mean (%)	Gain (%)
I am able to describe the structure of different 3D structure f plasmids.	69	88	19	81	100	19
I am able to describe interactions between DNA and small molecules.	69	81	12	69	100	31
I am able to perform common laboratory procedures used in the experiment.	75	100	25	94	100	6
I am able to interpret the electrophoresis gel results and relate them to the different 3D structures of plasmid DNA.	75	88	13	81	94	13
I am able to interpret the electrophoresis gel results and relate the different 3D structures of plasmid DNA.	50	88	38	44	94	50

[a] Agreement.

Table 3. Mean Scores for Students Who Took Both Laboratories

Statement	Agreement (%)
This experimental series has introduced to me one example of the biomedical applications of inorganic compounds.	94
This experimental series has enhanced my interest in learning the principles and methods in inorganic chemistry and molecular biology.	94
Performing this experimental series has helped me recognize how the knowledge and skills of different subject areas can be applied comprehensively in solving practical scientific problems.	94
This experimental series has helped me to understand the applications of inorganic chemistry.	94
This experimental series has helped me to understand the applications of molecular biology.	88

was important to use this staining method because it did not interfere with DNA unwinding. After the incubation of the plasmid with different concentrations of [(η^6-*p*-cymene)$RuOH_2(H_2N$-$CH_2CH_2NH_2$-*N*,*N*′)]PF_6, a change in the degree of supercoiling was observed in the electrophoresis gel. Exclusion of ethidium bromide was chosen for the following reasons. When ethidium bromide was used to visualize the DNA (added directly to the gel), it interfered with the migration of the supercoiled DNA because it could also bind and unwind DNA, which affected the correct interpretation of the results (Figure 6). In addition, it is important to mention that the use of Fast Blast DNA stain eliminated the use of toxic ethidium bromide.

The results of the experiment (Figure 5) clearly indicated that the Ru complex did unwind the DNA. From sample #1 to #8, as the concentration of the Ru complex increased, the mobility of the plasmid decreased, which indicated the decrease in the number of supercoils due to the unwinding of the DNA double helix. Sample #8 had the same mobility as the uncoiled plasmid, which indicated that the DNA plasmid was in the relaxed circular state. When the concentration of the Ru complex was further increased from sample #9 to #13, the mobility of the plasmid DNA increased again because further unwinding of the DNA double helix caused the plasmid to form supercoils in the opposite direction.

■ ASSESSMENT

An assessment plan that included formative assessment was employed. Three different assessments were performed: one for each module (Tables 1 and 2) and the other one (Table 3) was designed to assess whether the interdisciplinary series of experiments aided in the understanding of the concepts and techniques learned in the laboratories.

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At the time when both laboratories were taught, 4 students were taking both laboratories during the same semester, and the remaining students were enrolled only in one of the courses, either the advanced inorganic chemistry or the cell and molecular biology. Thus, there was an opportunity to examine if having an interdisciplinary series of experiments would be beneficial to student understanding. According to the results in Tables 1 and 2, for most of the questions (7 out of 10 questions), the gain of percent agreement is either higher or equal for the students who took both laboratories compared to the students who took only one laboratory. For the questions that have obtained equal responses from the two groups, the percent agreement of the post lab responses from the students who took both laboratories was either equal or very close to 100%; so the gain of percent agreement from this group could not have been higher. Finally, the mean percent agreement of the post lab responses was higher for the students took both laboratories than the ones who only took one laboratory. The assessment results for the students who were enrolled in both laboratories show that they benefited from having the interdisciplinary series of experiments (Table 3). Therefore, the students who took both laboratories not only gained more knowledge and skills that are specific for the individual laboratories, but also gained insight into how knowledge and skills from different subject areas can be combined effectively to solve real world problems.

■ CONCLUSIONS

This report shows that a successful interdisciplinary series of experiments was developed. In these experiments, students synthesized a complex in the advanced inorganic laboratory and they tested its biological properties in the cell and molecular biology laboratory. The assessment data demonstrates that students' understanding of the course materials and their applications were enhanced by having an interdisciplinary series of experiments.

■ ASSOCIATED CONTENT

Supporting Information

Student handout and notes for the instructor. This material is available via the Internet at http://pubs.acs.org.

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LABORATORY EXPERIMENT

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A One-Pot Self-Assembly Reaction To Prepare a Supramolecular Palladium(II) Cyclometalated Complex: An Undergraduate Organometallic Laboratory Experiment

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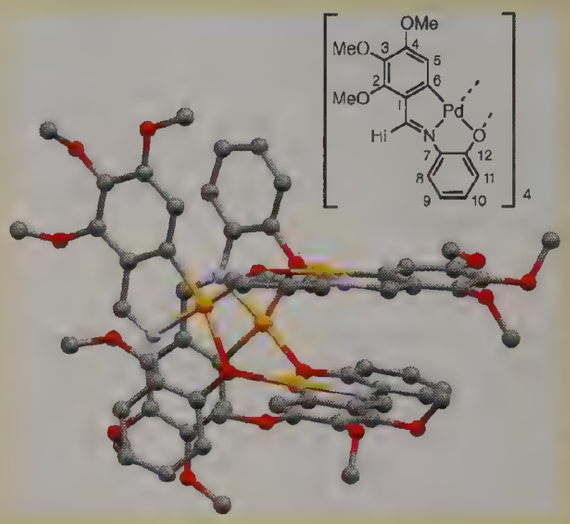
Supporting Information

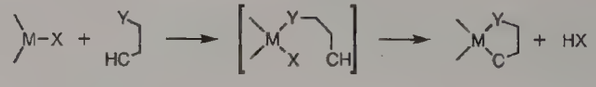
ABSTRACT: A laboratory experiment for students in advanced inorganic chemistry is described. Students prepare palladium(II) cyclometalated complexes. A terdentate [C,N,O] Schiff base ligand is doubly deprotonated upon reaction with palladium(II) acetate in a self-assembly process to give a palladacycle with a characteristic tetranuclear structure. This complex reacts with triphenylphosphine in a ligand-substitution process, cleaving of the tetranuclear structure. Students assign the resonances in the ^{1}H, ^{13}C-$\{^{1}H\}$, and ^{31}P-$\{^{1}H\}$ NMR spectra of the prepared compounds, whose 3D structure is given to understand the relative spatial disposition of the cyclometalated fragments. Mass spectra are interpreted taking into account the characteristic isotopic pattern originated by the tetranuclear $[Pd_4L_4]^+$ molecular ion.



KEYWORDS: Upper-Division Undergraduate, Inorganic Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Coordination Compounds, Mass Spectrometry, NMR Spectroscopy, Organometallics, Synthesis

One of the routes for the synthesis of supramolecular moieties is the self-assembly process where building blocks form an organized structure or pattern as a consequence of specific local interactions among the components, without external direction. In the classic sense, molecular units self-organize into ordered structures by weak noncovalent interactions, but in a broad definition, the assembly may be established through stronger interactions such as, for example, coordinate dative bonds.[1] Furthermore, cyclometalated complexes may be defined as a class of organometallic compounds in which an organic ligand is bonded to a metal center through a σ metal−carbon bond and a typical dative bond between a donor atom and the metal.[2] The compounds may be considered to be midway between the classic organotransition-metal complexes and coordination compounds, and consequently, they display the characteristic reactivity typical of both types of species, for example, ligand substitution reactions, insertion reactions into the metal−carbon bond, or catalytic activity toward the Suzuki or Heck reactions.[3] They are usually synthesized through the classical cyclometalation reaction, described by Trofimenko.[4] This is the direct reaction between the organic ligand and a transition-metal salt that yields the cyclometalated complex after C−H bond activation (Scheme 1). In the laboratory experiment reported herein, the student prepares a cyclometalated complex by reaction between the Schiff base ligand (Scheme 2)

Scheme 1. The Cyclometallation Reaction



M = Transition metal
X = Leaving group
Y = Donor atom

2,3,4-$(MeO)_3C_6H_2C(H)$=N[2-OHC_6H_4] and $Pd(OAc)_2$ (Scheme 3 and Figure 1).[5]

Because of the basicity of the acetate ions, the ligand is doubly deprotonated and coordinates to the palladium atom through a carbon atom, a C=N nitrogen, and a phenolate oxygen; subsequently, the Pd−ligand units self-assemble into tetrameric moieties through bridging phenolate oxygen atoms (Figure 1). Thus, cyclometalation and self-assembly both take place in a one-pot process. Cleavage of the tetranuclear structure is also carried out using a thermodynamically favorable ligand substitution reaction (Scheme 4 and Figure 2).

Laboratory projects to synthesize cyclometalated complexes related to bidentate [C,N] ligands to give dinuclear compounds

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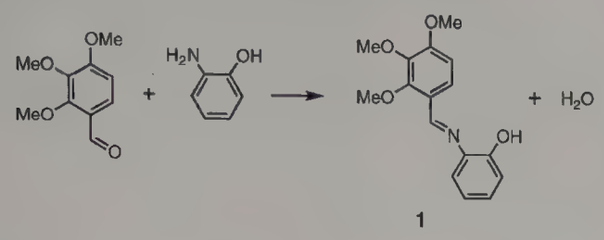
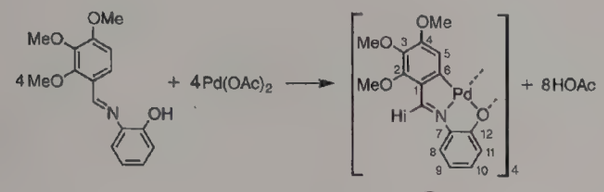
Scheme 2. Preparation of Ligand 1



1

Scheme 3. Preparation of the Tetranuclear Complex 2



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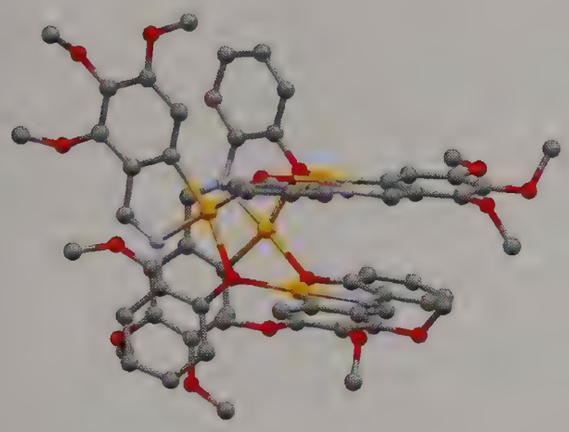
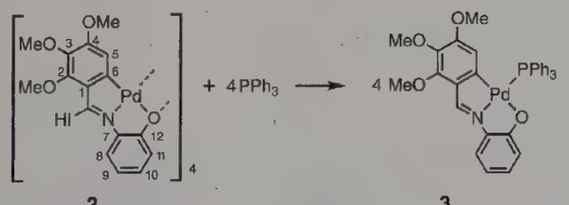


Figure 1. Structure of the tetramer **2**, $[Pd\{2,3,4\text{-}(MeO)_3C_6HC(H)=N[2\text{-}(O)C_6H_4)]\}]_4$: gray, carbon; red, oxygen; blue, nitrogen; yellow, palladium.

Scheme 4. Preparation of 3



2 3

have been previously described.[6] However, in the present case, the synthesis of a cyclometalated compound derived from a terdentate [C,N,O] ligand poses an example of the utility of self-assembly reactions to prepare supramolecular moieties, and therefore, new concepts must be used by the student. In addition, the synthetic procedure is different.

A number of important concepts are taught in this laboratory, including the synthetic procedures used in the preparation of the complexes and the study of their reactivity, for example, the laboratory setups required for a multistep synthesis, the manipulation of air sensitive compounds, vacuum filtration, recrystallization, or column chromatography in the final stages

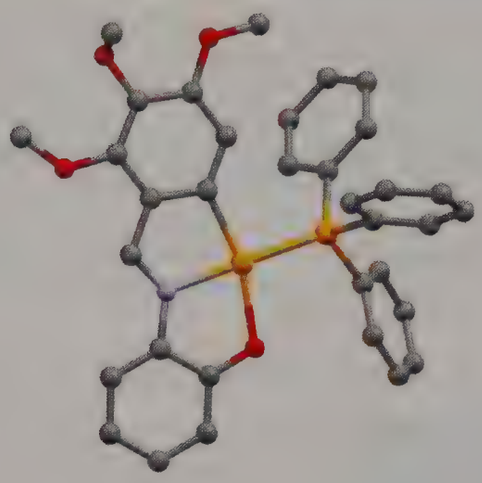


Figure 2. Structure of $[Pd\{2,3,4\text{-}(MeO)_3C_6HC(H)=N[2\text{-}(O)C_6H_4]\}(PPh_3)]$, **3**.

for purification of the complexes. The availability of molecular structures based on single crystal X-ray diffraction data for these complexes allows the student to be initiated in this technique. An intermediate level of coordination and organometallic chemistry knowledge is needed, and because both are normally taught in advanced inorganic and organic courses, the experiments depicted here are designed for second- or third-year undergraduates, or for those students who have taken at least a basic inorganic course as well as an initial laboratory in inorganic chemistry covering the basic synthetic and characterization techniques.

■ EXPERIMENT OVERVIEW

The experiment is designed to simulate the daily routine followed in a research laboratory; consequently, a specific synthetic problem is presented to the student who must first perform a literature search in order to find an adequate synthetic route for the complexes, which should also include the isolation and characterization procedures. The students are given prelab questions and instructions one week in advance of the laboratory sessions. They include the use of a scientific database and questions related to the synthesis and theoretical issues. Once this task is completed, a brief one-on-one discussion between instructor and student is conducted to gauge the level of preparation attained by the student. After this interaction, the student is given a detailed experimental procedure. The experimental work is carried out in four lab sessions of between one and four hours each. After the experiment is finished, students used proton and carbon NMR spectroscopy and mass spectrometry for the characterization of the final products; all the results then are given to the instructor.

■ EXPERIMENTAL DETAILS

The experimental procedure can be divided into three parts.

Preparation of $2,3,4\text{-}(MeO)_3C_6H_2C(H)=N[2\text{-}(OH)C_6H_4]$ (1)

The Schiff base ligand was prepared by a condensation reaction between 2-aminophenol and 2,3,4-trimethoxybenzaldehyde (Scheme 2) using chloroform as solvent and refluxing the solution for 24 h in a Dean−Stark apparatus (see Supporting Information). The ligand was isolated as a brown solid by evaporating the solvent in a rotatory evaporator and air-dried.

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Preparation of [Pd{2,3,4-(MeO)$_3$C$_6$HC(H)=N[2-(O)C$_6$H$_4$)]}]$_4$ (2)

Ligand **1** and palladium(II) acetate, in stoichiometric proportions, were suspended in degassed toluene and sealed under nitrogen, in a 25 mL Schlenk tube (Scheme 3). The mixture was heated with magnetic stirring at 60 °C for approximately 20 h, after which the flask was opened, the solution filtered, the solvent removed in a rotary evaporator, and the resulting red oil purified by column chromatography in silica gel. The final product was eluted as a red solid after elution with dichloromethane/ethanol (0.4%) and solvent removal.

Preparation of [Pd{2,3,4-(MeO)$_3$C$_6$HC(H)=N[2-(O)C$_6$H$_4$]}(PPh$_3$)] (3)

The tetranuclear complex was reacted with triphenylphosphine in acetone in a 25 mL Erlenmeyer flask for 1 h at room temperature with magnetic stirring (Scheme 4). Then, the solvent was removed in a rotary evaporator and the resulting solid recrystallized from acetone/hexane. The final product was separated as violet crystals after vacuum filtration.

■ HAZARDS

All reagents should be handled in a well-ventilated hood, with students wearing gloves, safety goggles, and lab coats. Toluene, *n*-hexane, ethanol, and acetone are highly flammable; dichloromethane and chloroform are toxic and irritant; 2,3,4,-trimethoxybenzaldehyde, palladium(II) acetate, and triphenylphosphine are irritants; 2-aminophenol is toxic and irritant.

The starting material, palladium(II) acetate, is expensive; however, in this microscale experiment, the amounts of reagents used are affordable. If necessary, palladium acetate can be regenerated easily following the method described by Granell et al.[6]

■ CHARACTERIZATION

The resonances in the ^{1}H NMR spectrum of Schiff base **1** can be assigned with the help of the instructor (see Supporting Information). In the ^{1}H NMR spectrum of the tetranuclear complex **2**, the resonance at 7.90 ppm corresponding to H6 (see labeling in Scheme 3) is absent as a consequence of metalation; thus, the H5 resonance is assigned as a singlet (5.67 ppm) instead of the doublet signal (6.78 ppm) in the spectrum of ligand **1**. One noticeable characteristic of the NMR spectrum is the high-field shift observed in the signals corresponding to the HC=N (8.98 ppm) and H5 (6.78 ppm) proton resonances; the low δ values are due to the tetranuclear structure of the complex, which puts the HC=N and H5 protons in the shielding zone of the phenyl rings of a neighboring metalated moiety. This effect is also observed in the resonance corresponding to the MeO group at C4, which appears at 2.92 ppm. To visualize the spatial disposition of the metalated ligands, the student may use an interactive 3D visualization program (most are freely available for the academic community). The coordinates of the atoms ate given in PDB files in the Supporting Information.

The most noticeable characteristics in the ^{13}C-{^{1}H} spectrum are the high-frequency shifts of the C6, C=N, and C1 resonances, as compared to the free ligands, due to formation of the metalated ring. The resonance assigned to the C−O carbon was shifted to higher frequency ca. 15 ppm consequent upon Pd−O bond formation.

The most noticeable feature in the ^{1}H NMR spectrum of complex **3** is the coupling of the H5 and HC=N resonances to the ^{31}P nucleus, appearing as doublets; J(PH) 3.9 and 10.2 Hz, respectively. The HC=N proton resonance, 8.17 ppm, shows a smaller low-field shift than in the parent cyclometalated complex **2**, in agreement with opening of the polynuclear structure. Surprisingly, the H5 resonance, 5.51 ppm, shows a greater shift than in **2** due to shielding by the phosphine phenyl rings; this shielding also affects the C4−MeO proton signal, which is also shifted to lower frequency upon phosphine coordination to the metal. The visualization of the 3D structure of the complex can also be of great help to the student in this case.

The mass FAB (fast-atom bombardment) spectra of **2** and **3** showed the clusters of peaks corresponding to the molecular ions. In the case of complex **2**, the characteristic pattern of a tetranuclear complex was found, whereas the spectra of **3** showed the expected pattern for a mononuclear ion. The student must understand the concept of isotopic pattern and calculate the relative intensities of the four most intense peaks corresponding to the molecular ions and compare them with the experimental values (alternatively the complete set of peaks corresponding to the molecular ion may be simulated and compared).

■ CONCLUSION

A convenient preparation for an air-stable self-assembled tetranuclear cyclometalated complex is described. The experiment uses equipment typically available in undergraduate laboratories. The students can identify the products using the standard spectroscopic techniques. This experiment provides an experimental entry point into the study of both coordination and organometallic complexes as well as the self-assembly process.

■ ASSOCIATED CONTENT

Supporting Information

Prelab student material including questions; student synthesis procedure; notes for the instructor including chemicals and equipment needed, hazards, and answers to student questions; a schematic of the Dean−Stark apparatus; NMR and mass spectra; PDB files. This material is available via the Internet at http://pubs.acs.org.

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LABORATORY EXPERIMENT

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A Physical Chemistry Experiment in Polymer Crystallization Kinetics

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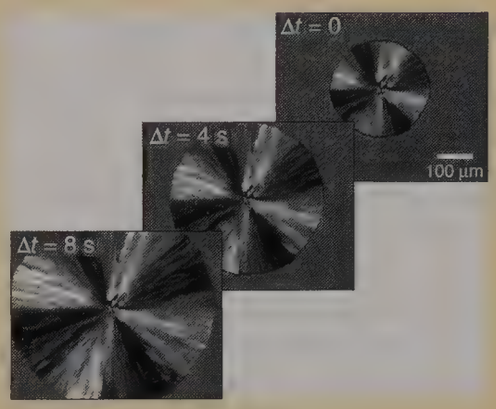
Supporting Information

ABSTRACT: A laboratory experiment currently used in an undergraduate physical chemistry lab to investigate the rates of crystallization of a polymer is described. Specifically, the radial growth rates of typical disc-shaped crystals, called spherulites, growing between microscope glass slides are measured and the data are treated according to polymer crystallization theory. The polymer used is a commercially available poly(ethylene glycol). Effects of molecular weight and crystallization temperature on the growth rate are demonstrated. Background information for students and instructor, experiment procedure, and full analysis of students' results are included in the Supporting Information.

KEYWORDS: Second-Year Undergraduate, Upper-Division Undergraduate, Laboratory Instruction, Physical Chemistry, Polymer Chemistry, Hands-On Learning/Manipulatives, Crystals/Crystallography, Kinetics, Materials Science



Polymer-related content is increasingly being added to the undergraduate chemistry program curriculum in many departments. An experiment has successfully been incorporated into the undergraduate physical chemistry lab in which the phase transition of linear chain polymers from the disordered melt to the ordered, semicrystalline solid is examined. Low molecular weight poly(ethylene glycol) (PEG) crystallizes at room temperature forming spherical crystals called *spherulites*. The spherulites can grow large enough under the right conditions to be visible with the naked eye if a thin film of melted polymer is left to solidify on a glass surface. Sandwiching a film of melted polymer between standard, glass microscope slides, however, forces the solid into disc-shaped spherulites. The radii of the spherulites increase linearly with time if the sample is kept isothermal.

This polymer crystallization experiment can be performed successfully using a polarized-light optical microscope equipped with a digital or video camera. (This is a standard piece of teaching equipment in most undergraduate geology departments and it may be feasible to borrow the equipment for the lab period.) Students measure the radial growth rates of disc-like crystals of polyethylene glycol and the results can be interpreted in terms of existing models of polymer crystallization kinetics. The experiment can be performed with or without the use of a temperature-controlled microscope hot stage, and both options are described with students' results. The experiment provides an opportunity to demonstrate and discuss the concepts of (i) nonequilibrium, kinetically controlled phase transitions; (ii) crystal growth; (iii) molecular weight effects on polymer crystallization; (iv) general polymer materials properties and applications; and (v) polarized light and birefringence. The experiment might also be adapted for use in a graduate-level course.

At least two articles in this *Journal* describe polymer crystallization from the melt state and both contain an in-depth treatment of the background theory including the origin of the birefringence in the polymer spherulites.[1,2] Those articles are recommended reading for the instructor. A brief background theory describing the structural hierarchy within the spherulite crystals and the equations used to describe their kinetics of crystallization, with references, is contained in the Supporting Information. This material is presented to students in the form of a reading assignment prior to the lab and a discussion is held on the subject as part of a prelab lecture and demonstration.

EXPERIMENTAL PROCEDURE

Equipment

Standard microscope glass slides and glass coverslips are used to prepare the sample. A hot plate with surface temperature of 65 °C is used to melt the PEG samples. As the temperature is relatively low, a simple beverage warmer plate (Radio Shack, Coffee/Beverage Warmer, model #61−8371) is used. A polarized-light microscope (Nikon Optiphot) is used to view the crystallization event. Typically, a 10X magnification lens is employed.

Either a video camera or a digital video camera is mounted onto the trinocular tube of the microscope so that the crystallization can be viewed in real-time using a television monitor or a computer projector, respectively. If a television monitor used, an overhead transparency sheet is placed onto the surface of the television screen, held in place by static electricity. A felt pen is used to make marks on the transparency to record the position of the growth front at regular time intervals. If digital video is used instead, then a whiteboard is used as a projection screen and students make their marks directly on the board.

The polymer can be crystallized at room temperature and therefore a temperature-controlled microscope hot stage is not

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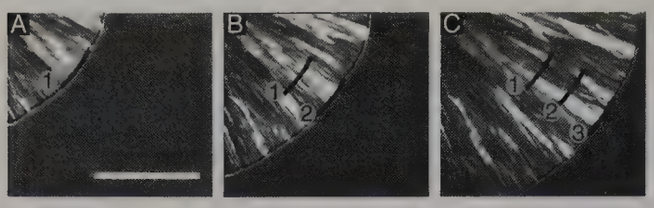

Figure 1. Time-lapse images as seen on the screen or whiteboard of a portion of a 4000 molecular weight PEG spherulite growing at 20 °C under the polarized-light optical microscope. Images taken at 2 s time intervals. Marks 1, 2, and 3 measure radial distances at the different times. Scale bar is 100 μm.

required. However, if such a hot stage is available, it can be used to investigate of growth rates at different temperatures above room temperature. In that case, any standard hot stage can be used. A Mettler FP-80 microscope hot stage was used for this purpose. It is a basic heating unit with no cooling option. Data from these experiments are provided in the Supporting Information.

Polymer Sample

Low molecular weight PEG was chosen for this experiment. The polymer is safe to handle on the open bench; is supplied as an easily handled grated wax so that associated problems with fine powders are avoided; possesses a relatively low melting point (60 °C); and crystallizes at room temperature at a rate that is easily measured. The polymer is commercially available in bulk quantities and relatively inexpensive. The three PEG polymers used in this experiment were donated by the former Union Carbide, and included three samples of Carbowax Sentry Poyethylene Glycol Flake with average molecular weights of 1450, 4600, and 8000, respectively. PEG has many diverse end-product applications that make it a good sample choice for discussion of material properties and end uses.

Sample Preparation

A standard glass microscope slide is preheated on a warm hot plate (set to about 65 °C). A crumb of polymer (less than a milligram) is placed on the slide, using tweezers, where it melts almost immediately. A glass coverslip is placed on top of the melted sample, causing the melted polymer to spread. There is no need to apply pressure on the top slide or coverslip, as the polymer naturally spreads quickly to form a small circle of melt film under the coverslip. The sample slide is then immediately removed from the hot plate and placed on the bench. It is not recommended to melt the sample at a higher temperature than 65 °C as this might lead to thermal degradation of the sample.

Using a micrometer, the thickness of the glass slide together with the top slide or coverslip can be measured before and after sample preparation to determine polymer film thickness, usually found to be in the range of 10−20 μm. Spherulite nucleation in thick polymer sample films is too dense and too rapid to obtain reproducible growth rate measurements. In polymer sample films that are too thin for measurement, there is insufficient contrast between the spherulites and the background melt for viewing. Spherulites growing at the very edge of the polymer sample film do not provide reproducible results and should be avoided.

Radial Growth Rate Measurements

The microscope polars are "crossed" so that no light is transmitted. The polymer sample slide is placed on the warm hot plate for about 10−30 s to remelt the sample. It is important

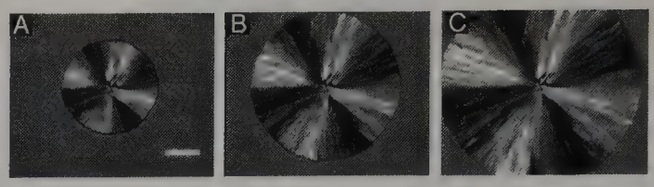

Figure 2. Micrographs of the Figure 1 sample isothermally crystallized at 20 °C under the polarized-light optical microscope, taken at (A) 200, (B) 204, and (C) 208 s after removal from the hot plate. Scale bar is 100 μm.

to just melt the sample and not permit it to flow, making it thinner. It is then quickly transferred to the microscope at room temperature or inserted into the hot stage on the microscope at the desired crystallization temperature. No light is transmitted until the birefringent spherulites appear.

Once a growing spherulite in the field of view has reached a reasonable size, a mark is made on the transparency (or whiteboard), tracing a portion of the observed outer edge of the growing spherulite (mark 1 in Figure 1A). Additional marks (2 and 3 in Figures 1B and 1C, respectively) are made at 1- or 2-s intervals until the spherulite growth is impinged by another spherulite or continues out of view. Students use an online metronome with an audible beat (e.g., set to 60 beats/min) to allow marks to be made at the desired frequency.

The growth of a single PEG spherulite at room temperature is shown in Figure 2. Keeping the center of the spherulite in view, the spherulite quickly grows out of the field of vision at a magnification useful for recording. A maximum number of radial growth measurements can be collected on a single growing spherulite by strategically placing the sample once a promising spherulite appears; the slide is moved so that the outer edge of the growing spherulite appears in one corner of the television screen (whiteboard) and grows toward the opposite corner. Reproducible growth rates can be recorded for a single sample that has been melted and crystallized a maximum of three times before discarding.

The radial distance is measured from the initial mark on the transparency (whiteboard) to subsequent marks until a minimum of five (but typically 12) radial measurements have been recorded. Radial distances can be converted using calibration measurements, made using a graduated micrometer slide, available with the microscope.

Each crystallization experiment at room temperature is complete in less than a minute. Sample preparation requires some practice to achieve a desired thickness. Students can expect to acquire plenty of data and have time to complete the data treatment of their results, all within a standard 3-h lab period.

■ HAZARDS

The polymer is safe to touch. Inhalation of any possible dust from the polymer should be avoided. Caution should be exercised around the hot plate and hot stage (if used). Hot glass slide sample assemblies should be handled with tweezers. Used microscope slides containing the PEG can be disposed of in a standard glass waste receptacle box. PEG can be disposed of in the regular trash.

■ RESULTS AND DISCUSSION

The radial distances are plotted against time. The data is fitted to a line. The slope is the spherulite radial growth rate of that

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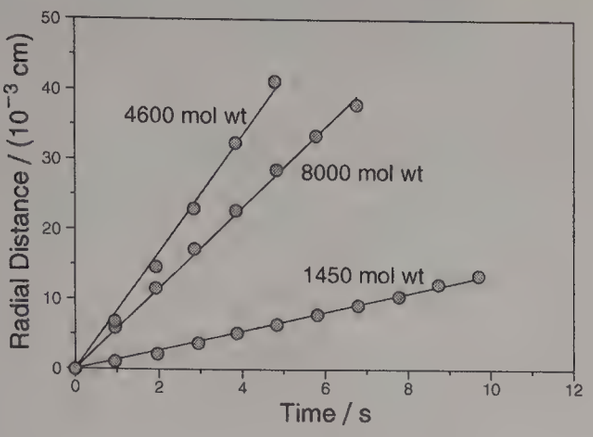

Figure 3. Radial distance vs time plots for PEG spherulites of different molecular weights. Samples crystallized at room temperature without hot stage. Time "zero" is the time the first mark is made on the transparency.

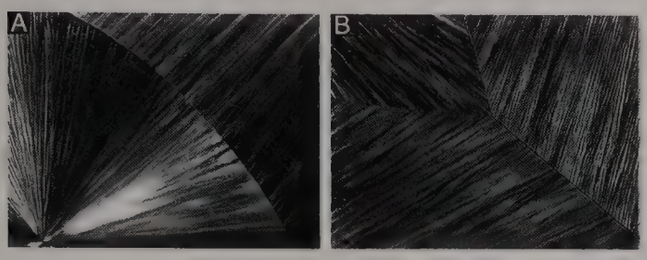

Figure 4. Images of impinged spherulites of PEG: (A) growing at different rates and (B) growing at the same rate.

molecular weight PEG sample at that temperature. The radial distance versus time plots for spherulites of the 4600, 8000, and 1450 molecular weights measured at room temperature (20 °C) are shown in Figure 3.

After the spherulites impinge and data collection is complete, the crystallized sample can be examined. Impingement lines appear where two spherulites have grown into contact with each other. Students may observe curved spherulite impingement lines, as shown in Figure 4A. Curved impingement lines result when the two impinging spherulites have grown at slightly different rates and are therefore an indication of a possible temperature gradient in the sample. Straight impingement lines indicate equal spherulite growth rates at the time of impingement (Figure 4B).

The students' data points plotted in Figure 5 show the effect of chain length on the radial growth rate of PEG samples at 20 °C. Each data point represents a single spherulite growth recording. The data points have not been averaged, showing the spread in growth rates that can typically be expected for each molecular weight. The results in Figure 5 show that radial growth rates measured under ambient conditions without the use of a thermally controlled microscope hot stage are reasonably reproducible.

The results in Figure 5 may or may not confirm the students' hypotheses regarding the effect of chain length on the rate of crystallization. It is important to note that the radial growth rate is a double exponential function, which depends on both the rate of transport of chains through the neat melt as well as nucleation events. In addition, the nucleation part of the function is twofold; the nucleation constant, K_g, in the Hoffman–Lauritzen growth rate equation[3] in the Supporting Information can be further defined into terms that depend on the attachment of the chain to

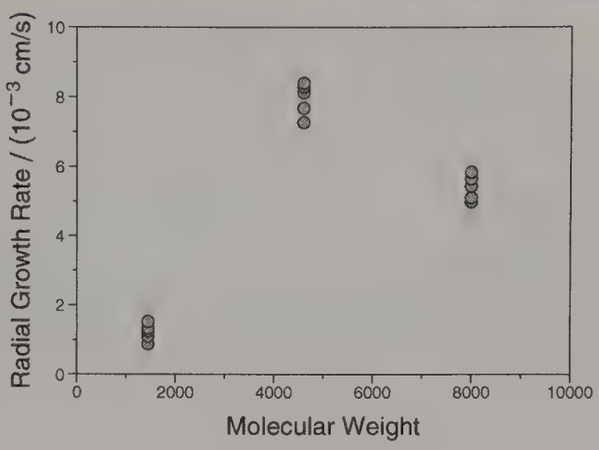

Figure 5. PEG spherulite radial growth rates at 20 °C as a function of polymer molecular weight.

the growing surface and on the subsequent folding of the chain to cover the surface, which is clearly affected by the chain length and the distribution of molecular weight in a single sample (i.e., the polydispersity).

Another notable feature among the different molecular weight samples crystallizing at room temperature is the induction time, the time required for spherulite nucleation to commence once the sample has been removed from the hot plate. Students may observe this qualitatively. Finally, observed morphological differences in the spherulites of different molecular weights might also be a topic for discussion.

The students' results from the variable temperature experiments are included in the Supporting Information. In this added information, student growth rate data was acquired for the 8000 molecular weight PEG sample at crystallization temperatures of 30, 32, 35, 37, 40, 43, 45, 48, and 50 °C. The students further analyzed the data by fitting it to the double-exponential growth rate equation. This level of data treatment might be used for the more advanced undergraduates.

■ CONCLUSION

This experiment has provided the physical chemistry students an opportunity to observe and discuss polymer crystallization, kinetically controlled phase transitions, and polymer materials in general. A successful experiment can be performed without the use of a temperature controlled microscope hot stage.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

A brief background theory describing the structural hierarchy within the spherulite crystals; the equations used to describe their kinetics of crystallization; data from variable-temperature experiments. This material is available via the Internet at http://pubs.acs.org.

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LABORATORY EXPERIMENT

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Theoretical and Experimental Study of the Primary Current Distribution in Parallel-Plate Electrochemical Reactors

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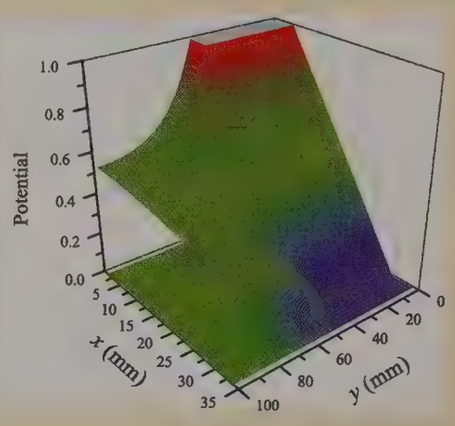
Supporting Information

ABSTRACT: A laboratory experiment is described to determine the primary current distribution in parallel-plate electrochemical reactors. The electrolyte is simulated by conductive paper and the electrodes are segmented to measure the current distribution. Experiments are reported with the electrolyte confined to the interelectrode gap, where the current distribution is uniform, and the case of unconfined electrolyte is treated by raising some segments. Experimental data are compared with theoretical results, and a close agreement is achieved. This experiment enables the student to gain a useful knowledge of the primary current distribution in electrochemical reactors.

KEYWORDS: Upper-Division Undergraduate, Chemical Engineering, Laboratory Instruction, Hands-On Learning/Manipulatives, Electrochemistry



Several authors[1−4] have analyzed the potential distribution and have claimed the necessity to design electrochemical reactors with uniform current distribution at the electrode surfaces to obtain better quality products, improve the utilization of electrode materials, avoid explosion risk, increase current efficiency, and achieve a better use of electrical energy. Current distribution depends on the geometric factors of the electrochemical reactor, the conductivity of the electrolyte, and the kinetics of the electrochemical reactions. According to these variables, current distribution is classified as primary when only geometric factors are considered. Taking into account kinetics, current distribution is called secondary when the reaction is under charge transfer control, and tertiary when mass transfer is included in the analysis. The primary current distribution represents the simplest situation for mathematical treatment and in monopolar reactors it shows the most pronounced case. Thus, for a given electrochemical system, if the primary current distribution is acceptable, the secondary and tertiary ones are still better. For these reasons, the primary current distribution is frequently analyzed.

Electrochemical reactors with parallel-plate electrodes are the more common cell arrangement used in the industrial practice,[5] that is, electrowinning and electrorefining of metals, electrosynthesis of inorganic and organic compounds, fuel cells, and batteries. These reactors present, as an advantage, simplified constructive features; the interelectrode gap is uniform and can be defined by a single and easily adjustable geometric variable. Moreover, the primary and secondary current distributions are substantially uniform over most of the central portion of the electrode. However, a considerable edge effect occurs near the inlet and outlet of the electrolyte to the electrode region, where the current density tends toward very high values. Furthermore, Frias-Ferrer et al.[6] planned a laboratory experiment to examine the fluidodynamic aspects of the entrance and exit effects in parallel-plate electrochemical reactors.

The aim of the present contribution is to describe a laboratory experiment for a course of electrochemistry to introduce students to the study of current distribution in electrochemical systems and to compare experimental data with theoretical results from the numerical and analytical solution of the Laplace equation. This laboratory experiment was carried out by advanced students of chemical engineering during the last four years. It was performed in approximately 2 h by groups of two students and it provided the students with an overall understanding of primary current and potential distribution in electrochemical systems. The students presented a full report about the experiment a week later at the end of the class. The report contains the experimental procedure, a brief summary of the theory, discussion of the results, and a comparison between experimental and theoretical results.

EXPERIMENTAL DETAILS

Description of the Experimental Setup

The experimental arrangement is shown in Figure 1 and the symbols are defined in Table 1. The electrolyte was simulated by a sheet of conductive paper (Pasco Scientific, PK 9025)[7]

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mounted on a nonconducting board and the electrodes were formed by copper segments, 6.1×10^{-3} m wide, at opposite sides of the conductive paper. The segments were insulated from one another by an approximately 5×10^{-4} m thick Teflon slide and were connected to the conductive paper by colloidal silver paint (Pasco Scientific, PK-9031B) to minimize the contact resistance. The thickness of the conductive paper was 1.3×10^{-4} m and the resistivity was 3.48 Ω m. Calibrated resistors, approximately 30 Ω resistance, were intercalated between each segment and the current feeder of the electrode. The effect of the calibrated resistors on the current distribution can be neglected due to the small value of their resistance in comparison to that of the conductive paper. A dc power supply was used to apply a constant current to the feeders. The electric connection was made in the middle point of the current feeder of each electrode. Two electrochemical systems were examined. In the first one, the conductive paper and 15 copper segments were trimmed so that they were the same length, 0.1 m with 0.02 m interelectrode gap. This case, called confined electrolyte and represented in Figure 1A, permits for, by pressing the segments, the adjustment of the contact resistance with the conductive paper to obtain a uniform current distribution. In the second system, some terminal segments were raised. Thus, the paper length is larger than the electrode length, represented as unconfined electrolyte in Figure 1B.

Experimental Procedure

Before each laboratory session, to facilitate the student work and shorten the duration of the experiments, instructors should calibrate the resistors. The instructors should also verify that the contact resistance between each segment and the conductive paper is minimal and that it approximately has the same value for all segments, following the guidelines given in the Supporting Information.

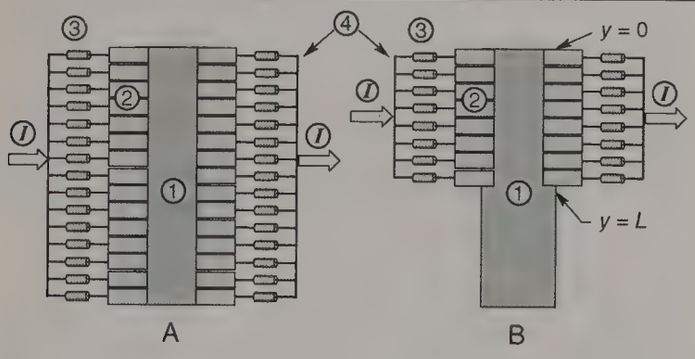

Figure 1. Schematic view of the experimental arrangement for (A) confined system and (B) unconfined system: (1) conductive paper, (2) segmented electrodes, (3) calibrated resistors, (4) current feeder of the electrode.

The experimental current distribution at each electrode is determined by measuring the ohmic drops in the calibrated resistors. The data acquisition is performed using a computer controlled analogue multiplexer. An alternative procedure is to connect the calibrated resistors to a selector switch with 15 positions coupled to a recorder. For a given value of the total current, several experiments are carried out to verify the reproducibility of the results. Typical values of the applied potential to the system are in the range from 5 to 40 V to obtain a total current between 1 and 5 mA.

The next step is to compare the experimental results with the theoretical calculations obtained by solving the Laplace equation.

■ THEORETICAL CONCEPTS

The primary current distribution in electrochemical reactors is obtained by solving the Laplace eq 1 in the solution phase

$$\nabla^2 \phi = 0 \tag{1}$$

with the following boundary conditions at the terminal anode,

$$\phi = U_A \tag{2}$$

at the terminal cathode,

$$\phi = U_C \tag{3}$$

and at the insulating walls

$$\left.\frac{\partial \phi}{\partial x}\right|_{\text{inslulating walls}} = \left.\frac{\partial \phi}{\partial y}\right|_{\text{insulating walls}} = 0 \tag{4}$$

The current density at any point at the electrode surface is given by

$$j_i = -\left.\frac{1}{\rho}\frac{\partial \phi}{\partial x}\right|_{\text{at electrode i}} \quad \text{with i = A or C} \tag{5}$$

Numerical Resolution of the Laplace Equation

The numerical resolution of the Laplace equation for the unconfined system shown in Figure 1B is outlined in the following paragraphs. The geometric parameters are defined in Figure 2. Therefore, the solution of eq 1, by the finite difference method with an equidistant grid,[8] yields the potential for the bulk of the electrolyte:

$$\phi(x,y) = \frac{\phi(x+h,y) + \phi(x-h,y) + \phi(x,y+h) + \phi(x,y-h)}{4} \tag{6}$$

Table 1. Description of the Symbols

Symbol	Description	Symbol	Description
a_i	Constants in eq 15	U	Potential of the metal phase (V)
e	Interelectrode gap (m)	x	Axial coordinate (m)
h	Distance between two nodes in the potential grid (m)	y	Axial coordinate (m)
H	Distance from the electrode end to the reactor bottom (m)	ε	Dimensionless geometric parameter given by eq 19
j	Current density (A m^{-2})	ρ	Electrolyte resistivity (Ω m)
j_{mean}	Mean current density (A m^{-2})	ϕ	Potential in the solution phase (V)
L	Electrode length (m)	A	Anode
r	Electrolyte thickness outer the interelectrode gap (m)	C	Cathode

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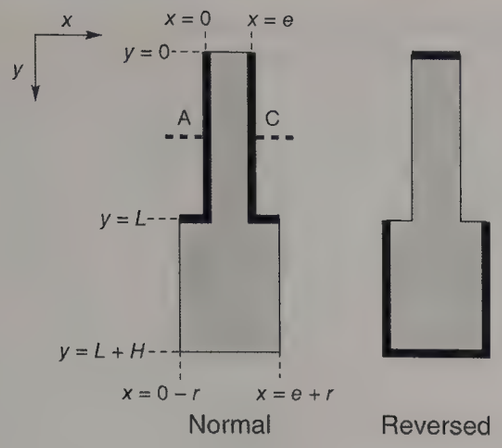

Figure 2. Geometric representation of an electrochemical reactor with unconfined electrolyte to the interelectrode gap. Thick black lack lines, electrodes; light gray shading, electrolyte; A, anode; C, cathode. Left normal arrangement. Right inverted arrangement.

The finite difference method applied to the boundary condition given by eq 4 produces

For $x = 0 - r$ and $L < y < L + H$ is

$$\phi(x,y) = \frac{2\phi(x + h,y) + \phi(x,y + h) + \phi(x,y - h)}{4} \quad (7)$$

For $x = e + r$ and $L < y < L + H$ is

$$\phi(x,y) = \frac{2\phi(x - h,y) + \phi(x,y + h) + \phi(x,y - h)}{4} \quad (8)$$

For $y = 0$ and $0 < x < e$ is

$$\phi(x,y) = \frac{\phi(x + h,y) + \phi(x - h,y) + 2\phi(x,y + h)}{4} \quad (9)$$

For $y = L + H$, $0 - r < x < e + r$ is

$$\phi(x,y) = \frac{\phi(x + h,y) + \phi(x - h,y) + 2\phi(x,y - h)}{4} \quad (10)$$

Likewise, the potential in the corners of the electrolyte is given by

For $y = L + H$ and $x = 0 - r$ is

$$\phi(x,y) = \frac{2\phi(x + h,y) + 2\phi(x,y - h)}{4} \quad (11)$$

For $y = L + H$ and $x = e + r$ is

$$\phi(x,y) = \frac{2\phi(x - h,y) + 2\phi(x,y - h)}{4} \quad (12)$$

Moreover, the electrodes are represented by

For $x \leq 0$ and $0 \leq y \leq L$ is

$$\phi(x,y) = U_A \quad (13)$$

and

For $x \geq e$ and $0 \leq y \leq L$ is

$$\phi(x,y) = U_C \quad (14)$$

To calculate the current density for a given axial position y along the electrode length, the four potential points in the solution phase nearest to each electrode surface, that is, $\phi(h, y)$, $\phi(2h, y)$, $\phi(3h, y)$, and $\phi(4h, y)$, were fitted with the polynomial:[9]

$$\phi(x,y) = a_0 + a_1x + a_2x^2 + a_3x^3 \quad (15)$$

Introducing the first derivative of eq 15, evaluated at the electrode surface, in eq 5 the current density is given by

$$j(y) = -\frac{1}{\rho}\frac{\partial\phi(x,y)}{\partial x}\bigg|_{\text{at electrode}} = -\frac{a_1(y)}{\rho} \quad (16)$$

Thus, the current density distribution results in

$$\frac{j(y)}{j_{\text{mean}}} = \frac{a_1(y)}{\frac{1}{L}\int_0^L a_1(y)\,dy} \quad (17)$$

The current lines are obtained solving the potential distribution for the inverted arrangement,[10] sketched on the right-hand side of Figure 2, where the electrodes are replaced by insulating walls, whereas the insulating walls of the normal configuration are considered electrodes. Thus, the equipotential lines of the inverted cell are equivalent to the current flow lines of the normal cell.

The students can calculate the theoretical primary potential and current density distributions with an Excel spreadsheet included in the Supporting Information.

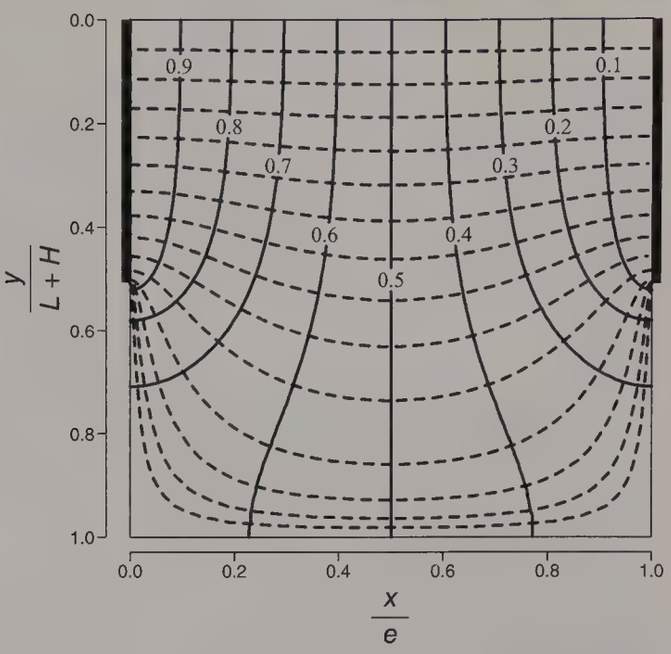

Figure 3. Contour plots of potential distribution and current lines for an unconfined system. $L = H$, $e = L + H$, $r = 0$. Full lines: potential distribution. Dashed lines: current lines. Thick black lines: electrodes. $U_A = 1$ V. $U_C = 0$ V.

Analytical Solution of the Laplace Equation

Two plane electrodes placed opposite to each other in the walls of a channel flow of infinite length has been theoretically considered by Parrish and Newman.[11] In this case, the following equation represents the primary current distribution

$$\frac{j(y)}{j_{\text{mean}}} = \frac{\varepsilon\cosh(\varepsilon)/\mathrm{K}[\tanh^2(\varepsilon)]}{\sqrt{\sinh^2(\varepsilon) - \sinh^2(y\varepsilon/L)}} \quad (18)$$

where $\mathrm{K}(m)$ is the complete elliptic integral of the first kind and ε is a characteristic geometric parameter given by eq 19. In eq 18, the y coordinate is measured from the center of the electrode

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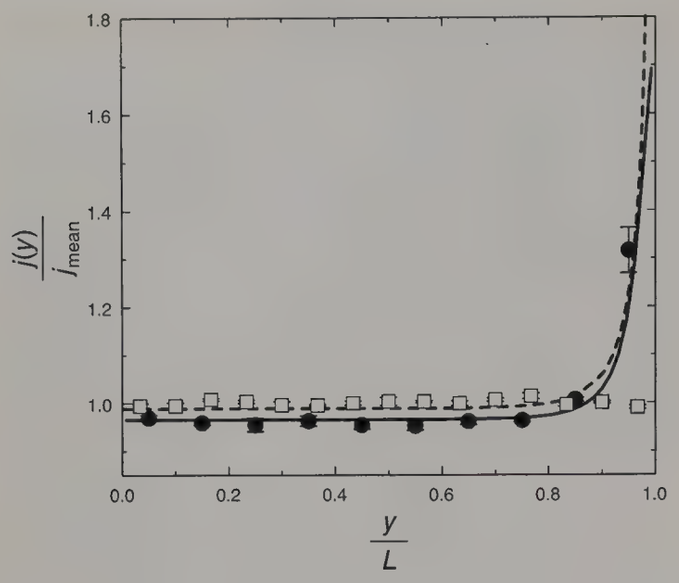

Figure 4. Current distribution: (□) confined system, (●) unconfined system with 10 segments. Vertical bars: standard error of the mean. Full line: numerical solution of the Laplace equation, r = 5 mm. Dashed line: current distribution according to eq 18.

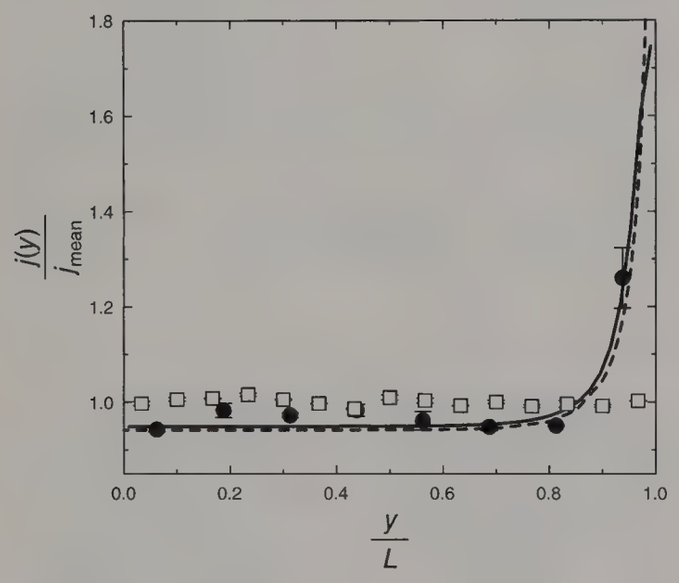

Figure 5. Current distribution: (□) confined system, (●) unconfined system with 8 segments. Vertical bars: standard error of the mean. Full line: numerical solution of the Laplace equation, r = 7 mm. Dashed line: current distribution according to eq 18.

with a total electrode length of $2L$.

$$\varepsilon = \frac{\pi L}{e} \tag{19}$$

The experimental results are compared with the theoretical calculations obtained solving the Laplace equation with the numerical procedure previously explained or given by eq 18.

■ RESULTS AND DISCUSSION

The potential distribution and the current lines for an unconfined system, obtained by numerical solution of the Laplace equation, are shown in Figure 3. A symmetrical potential distribution is observed and the current lines are concentrated on the lower edge of the electrodes because of the excess of electrolyte in this region.

Experimental results of unconfined systems with 10 and 8 segments, respectively, are shown in Figures 4 and 5. Each point represents the mean value at both electrodes for three experiments with different currents; the standard error of the mean is given by the vertical bars, and the full line was obtained by numerical solution with the finite difference method of the Laplace equation. The results of the confined system are also included. As expected, the unconfined system presents a pronounced current distribution and there is a close agreement between experimental and theoretical results. The dashed lines in Figures 4 and 5 correspond to the behavior according to eq 18, where a more pronounced current distribution is shown because the unconfined electrolyte is larger in this theoretical treatment.

From Figures 4 and 5, it is concluded that the presence of electrolyte outside the interelectrode gap produces a pronounced current distribution that may compromise the performance of the electrochemical reactor. The solution to obtain a uniform current density distribution is to enclose the electrodes with perpendicular insulating plates as shown the confined system.

■ HAZARDS

Equipment and materials used in this work do not present risks to people or the environment.

■ CONCLUSION

This laboratory experiment allowed students to obtain an improvement in understanding of the primary current and potential distribution in electrochemical systems, which is very important in electrochemical technology and also in basic electrochemistry. During the exercise, students clearly observed that the presence of unconfined electrolyte in an electrochemical reactor produces current distribution at the electrode surfaces. Students corroborated the theoretical results, according to the numerical solution of the Laplace equation, with the experimental ones and they also compared theoretical and experimental results with the current distribution according to a model of electrochemical reactor. The final student laboratory reports were of high quality.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Instructions for students, notes for the instructor; and an Excel spreadsheet to calculate the theoretical primary potential and current density distributions. This material is available via the Internet at http://pubs.acs.org.

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A Simple and Low-Cost Ultramicroelectrode Fabrication and Characterization Method for Undergraduate Students

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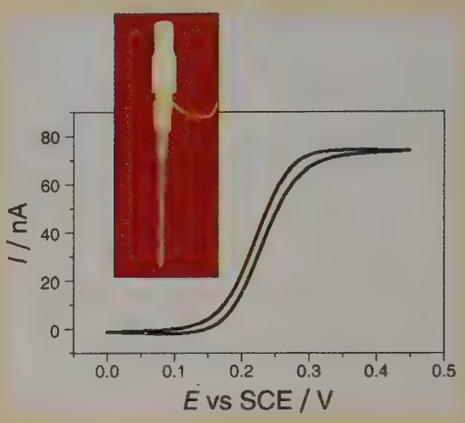
Supporting Information

ABSTRACT: A laboratory experiment is described in which students fabricate disk-shaped gold and platinum microelectrodes with diameters of 10−50 μm by sealing sodalime glass with metal microwires. The electrodes are characterized by performing cyclic voltammetry in aqueous and acetonitrile solution. Commercial microelectrodes are expensive (cost depends on the diameter of electrode) and may deter introduction of voltammetric experiments into laboratory classes at the undergraduate level. The students also fabricate a simple operational amplifier (op amp)-based potentiostat along with a low-current measuring device. This low-cost fabrication helps the students understand the details of instrumentation of a potentiostat with special knowledge in analog electronics. This experiment can be included in the electrochemistry laboratory course for undergraduate students. This experiment can also be incorporated into various undergraduate laboratory courses such as analytical chemistry, general chemistry, biochemistry, physical chemistry, and instrumentational methods of analysis.



KEYWORDS: Second-Year Undergraduate, Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Physical Chemistry, Hands-On Learning/Manipulatives, Electrochemistry, Laboratory Equipment/Apparatus, Oxidation/Reduction

Ultramicroelectrodes are electrodes with at least one of the dimensions of the order of micrometer or less. Initially, microelectrodes were used for biological and medical research. In early 1980s, Fleischmann and his co-workers at the Southampton Electrochemistry group exploited the versatile properties of microelectrodes in electrochemical studies. The ultramicroelectrodes, owing to their extremely small size, have certain unique characteristics that make them ideal for studies involving high-resistive media, high-speed voltammetry, and in vivo electrochemistry in biological systems.[1] They exhibit a high rate of mass transfer combined with a low ohmic drop and double-layer charging current, which make them a powerful tool for studies of fast heterogeneous kinetics at low concentrations. Owing to the small electrode area, the currents associated with microelectrodes are small, of the order of pA to nA, and the current densities are high.

Microelectrodes make it possible to carry out experiments that are not possible using conventional-sized macroelectrodes. This is due to the considerable difference in electrochemical responses between micro- and macroelectrodes. Because of the small area of microelectrodes, the double-layer capacitance is considerably reduced relative to macroelectrodes. This allows the electrode potential to be changed rapidly, which can be utilized in voltammetric measurements in submicrosecond time scale.[2,3] At normal time scales, cyclic voltammograms obtained with ultramicroelectrodes are different from those of macroelectrodes. The voltammograms are sigmoidal-shaped, analogous to the S-shaped polarograms obtained with dropping mercury electrode or rotating disk electrodes. The rate of mass transport (diffusion) plays an important role in defining the shape of voltammograms. At normal-sized macroelectrodes, the mass transport occurs mostly perpendicular to the electrode surface (planar diffusion). The result is a typical peak-shaped voltammogram for the macroelectrodes. For a reversible redox process, the peak current follows Randles−Sevcik equation[4,5]

$$I_p = (2.69 \times 10^5) n^{3/2} C^* D^{1/2} \nu^{1/2} \quad (1)$$

in which I_p is the peak current density (A/cm^2), n is the electron stoichiometry, D is the diffusion coefficient (cm^2/s) of the electroactive species, C^* is the bulk concentration of the electroactive species, and ν is the scan rate (V/s). On the other hand, for a microelectrode, an S-shaped voltammogram is obtained at lower scan rates, which changes into a peak-shaped one at high scan rates. At lower scan rates, the rate of electrolysis is almost equal to the rate of diffusion, which takes place in a hemispherical fashion as a time-independent process. Hence, it produces steady-state S-shaped voltammogram. At higher scan rates, the rate of electrolysis exceeds the rate of diffusion to a larger extent. At such a fast time scales, redox species take longer time to diffuse. Hence, the current changes with time, and peak-shaped voltammograms are obtained.

The diffusion processes occurring at the ultramicroelectrodes can be understood by solving the diffusion problems for dropping mercury electrodes used in traditional polarography. For a

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spherical electrode (radius r), current is given by

$$i_{\text{spherical}} = nFADC^*[1/(\pi Dt)^{1/2} + 1/r] \quad (2)$$

where $A = 4\pi r^2$ is the area for a spherical electrode and F is the Faraday constant. According to this equation, at longer time scales, the current should be time independent. For a macroelectrode, the current due to semi-infinite planar diffusion is given by the Cottrell equation

$$i_{\text{planar}} = nFADC^*/(\pi Dt)^{1/2} \quad (3)$$

that is,

$$i_{\text{spherical}}/i_{\text{planar}} = 1 + [(\pi Dt)^{1/2}/r] \quad (4)$$

The parameter $(Dt)^{1/2}/r$ will determine when the current will be essentially constant in nature. The expression $i = 4nFDCr$ correlates the steady-state current to the electrode radius r of a microelectrode. Hence, one can determine the dimension of a microelectrode by directly measuring the steady-state current from the S-shaped voltammogram, provided the value of D is known. For a redox reaction with unknown D value, the above expression can be used to determine the diffusion coefficient D. Because the steady-state current is proportional to the bulk concentration of electroactive species, the above expression can also be used in determining unknown concentration of some electroactive species.

Microelectrodes of different geometries such as disks, rings, and bands are commonly found in the literature.[2,5] The fabrication and use of microelectrodes had received little attention in the undergraduate laboratory courses in chemistry as seen from the citation of papers in this *Journal*. The article titled "Electrochemistry at Nanometer-Scaled Electrodes"[6] is the most recent and significant one found in this *Journal*.

Ultramicroelectrodes are available commercially from most companies specializing in electrochemical apparatus, but the cost is high and electrodes of variable diameters are not available. For example, the price of 10 μm diameter platinum and gold microelectrodes from a well-known electrochemical instrument supplier is about U.S. $400. In this article, a simple method of fabricating disk-shaped and inexpensive gold and platinum microelectrodes for undergraduate electroanalytical labroratory course is described. Although, the approach is well-known in various electrochemistry research laboratories, it has not been introduced in undergraduate laboratory programs in chemistry. The electrodes are characterized by performing cyclic voltammetry in an aqueous and a nonaqueous (acetonitrile) solution.

■ MICROELECTRODE FABRICATION

The main goal of this laboratory experiment is the fabrication of inexpensive microelectrodes of different diameters by sealing glass with metal microwires of required diameters. A wide variety of methods are available for constructing ultramicroelectrodes.[2] Disks, bands, and rings are some of the common geometries of ultramicroelectrodes. The ultramicroelectrode disk is easiest to fabricate and can be made by encapsulating metal microwire in a matrix of glass or epoxy. Stanton et al.[7] described the fabrication of ultramicroelectrodes by sealing microwires into epoxy. Glass is the best choice to be embedded with metal, as it has the properties of good insulation, transparency, inertness, and is easy to polish and seal with metals. Perfect metal-to-glass sealing is an important step in modern scientific glass blowing. To ensure a satisfactory seal, glass must wet the surface of the metal, and the metal must have a coefficient of thermal expansion similar to that of the glass. This will give perfect glass-to-metal sealing without any leakage. Platinum and gold have thermal expansion coefficients of 91×10^{-7} and 143×10^{-7} °C^{-1}, respectively, and the soft glass used here has a thermal expansion coefficient of 92×10^{-7} °C^{-1}. The glass value is suitable for platinum due to the similar thermal expansion coefficients. Although the thermal expansion coefficient of our glass is almost two-thirds that of gold, excellent glass−metal seals have been achieved and fabricated gold microelectrodes show no leakage even when kept in electrolytes for days.

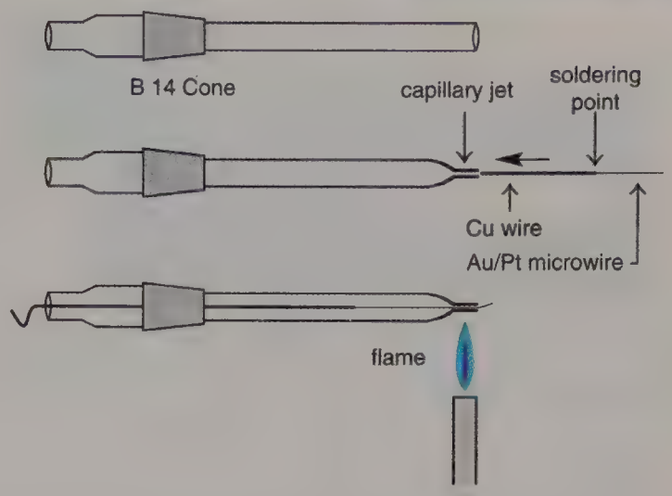

Figure 1. Steps involved in the fabrication of microelectrode. The heavy arrow depicts the direction of insertion of the wire.

A sodalime lamp glass, which is commonly known as soft glass or lamp glass,[8] is available from the fabricators of commercial fluorescent lights. It has a softening point of 700 °C and high percentage of sodium monooxide. The general composition of sodalime lamp glass, from the manufacturers, is SiO_2, 73.5%; Na_2O, 16.3%; CaO, 4.7%; K_2O, 0.3%; Al_2O_3, 1.6%; Sb_2O_3, 0.17%; MgO, 3.4%; and FeO, 0.03%. Similar kinds of soft glasses whose thermal expansion coefficient is close to that of the metals to be sealed may also used.

The following method achieved a perfect metal−glass seal; the steps for the fabrication of ultramicroelectrode are illustrated in Figure 1. The gold and platinum microwires (diameters 10, 12.5, 40, and 50 μm) were obtained from Advent Research Materials, (Oxford, U.K.). A 10 cm length of gold or platinum microwire was soldered with a thin copper wire for electrical contact without any "blob" at the soldered connection, as any blob causes obstruction when drawing the soldered microwire through a narrow capillary jet. The length of the microwire depends on the height of the electrochemical cell along with the cap. The soldered gold or platinum wire was inserted carefully through the fine capillary jet and drawn to the other side and positioned. The wire end just protruded outside the jet capillary. A pinpoint low-temperature flame was set at the burner and the capillary tube was sealed with the gold or platinum wire. High-temperature, broad-flame, and prolong heating may spoil the wire and conduction of heat may sometimes desolder the wire. The sealing must take place from one end progressively to the other end to avoid air trapping between the glass and metal interface, which will weaken the sealing. The other end of the soldered copper wire was folded at the top of the cone and was used as the external electrical contact. However, sealing ultramicroelectrode wires

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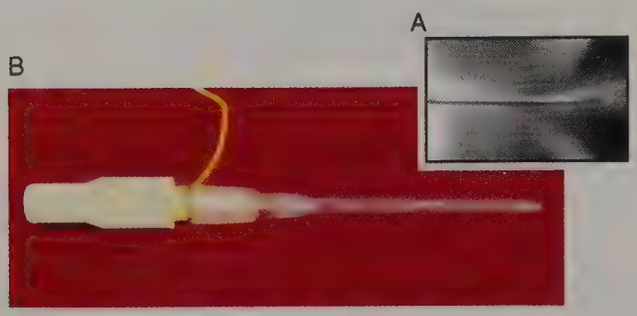

Figure 2. (A) Photomicrograph of the gold microwire–glass seal taken at the tip end of the microelectrode. (B) Photograph of the microelectrode fabricated in the laboratory.

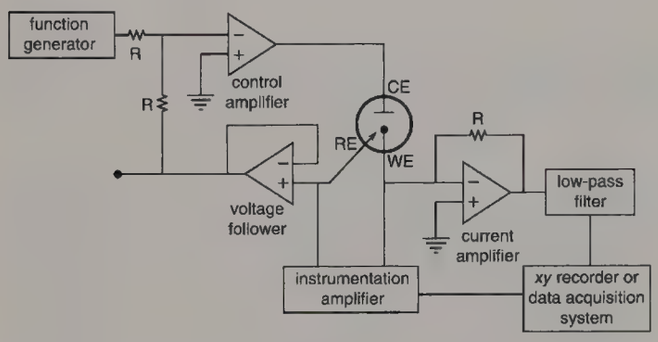

Figure 3. Block diagram of the two amplifier potentiostat used for microelectrode studies.

into glass is not simple and the undergraduate students needed several trial runs before fabricating a usuable ultramicroelectrode. The electrode tip was rendered to disk-shaped by grinding in 1000 grade carborandum, 1500 grade emery paper, and the resulting electrode was smoothed by polishing on a microcloth having a slurry of 1.0, 0.3, and 0.05 μm alumina powder (Buehler) successively. A photomicrograph of the gold microwire–glass seal at the tip end of the fabricated microelectrode is shown in Figure 2A and the photograph of the microelectrode fabricated in our laboratory is shown in Figure 2B. The metal–glass sealing portion was examined under the microscope for any imperfections, although the quality of the electrode should ultimately be assessed electrochemically by cyclic voltammetric studies of standard redox couples such as ferrocyanide/ferricyanide in aqueous media and ferrocene/ferricenium in acetonitrile. From this procedure, many electrodes can be produced with costs on the order of U.S. $5–10 per electrode.

■ INSTRUMENTATION

Cyclic voltammetry was carried out using a homemade potentiostat and also with an EG&G potentiostat (model 263 A) interfaced to a PC through a GPIB card (National Instruments). The fabrication of a simple operational amplifier (op amp)-based potentiostat along with the low-current measuring device can be accomplished by the students.[9] These amplifiers are inexpensive and have excellent sensitivity. The currents normally associated with microelectrodes are of the order of pA to nA. The problems associated with measurement of small currents and elimination of noise can be overcome by the use of ultra low noise, low bias current amplifiers. Though the current amplifier output can be recorded using any inexpensive *xy* recorder, a simple USB-based data acquisition system is recommended for data transfer to the PC and subsequent display. An SRS function generator

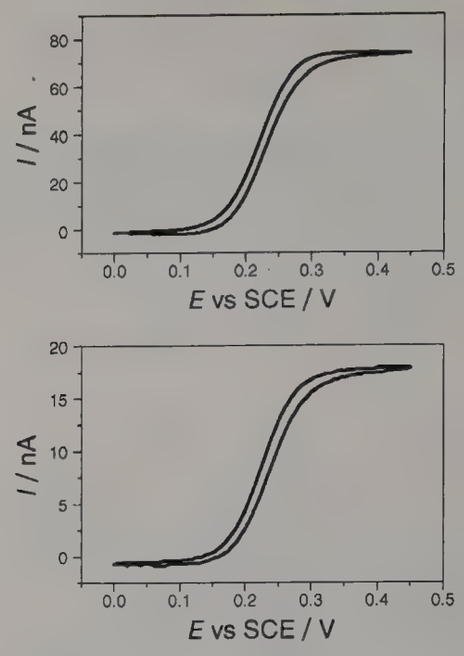

Figure 4. Cyclic voltammograms for 10 mM ferrocyanide with 1 M NaF with (A) 40 μm Au and (B) 10 μm Pt microdisk electrodes. Scan rate: 5 mV/s.

(model DS340, 15 MHz) was used as a voltage source for supplying suitable voltage ramp. The acquired data can be plotted on line using Labview or similar graphical software. The schematic diagram of the experimental setup used for microelectrode studies is shown in Figure 3.

■ HAZARDS

Acetonitrile may be fatal if swallowed, inhaled, or absorbed through skin; may cause irritation to skin, eyes, and respiratory tract; and is flammable. TBAFB causes irritation, may be harmful if swallowed, and is combustible. Ferrocyanide, ferrocene, and sodium fluoride are extremely hazardous in case of ingestion. Gloves and safety glasses should be worn throughout the experiment. Care must be taken when using the burner to seal the glass.

■ EXPERIMENT

The experiment including the fabrication of ultramicroelectrodes along with the design of the experimental setup can be completed in three weeks. Students performed this experiment in 4-h lab sessions that met three times a week. The students reported their results in the laboratory notebook. Students worked in groups of 5 and 50 students contributed to the data presented here.

■ CHARACTERIZATION OF MICROELECTRODE BY CYCLIC VOLTAMMETRY

Platinum, 10 and 50 μm diameter, and gold, 12.5 and 40 μm diameter, microdisk electrodes were fabricated. The electrodes were characterized by performing cyclic voltammetry (CV) of 10 mM potassium ferrocyanide with 1 M NaF in water and also of 1 mM ferrocene in 0.1 M TBAFB/acetonirile solution (TBAFB = tetrabutylammonium tetrafluoroborate). For a disk-shaped microelectrode, a typical sigmoidal voltammogram was observed and the limiting plateau current from CV is given by

$$i_{lim} = 4nFrC^*D \quad (5)$$

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Table 1. Calculated Radius Values for Microelectrodes from Sigmoidal-Shaped Steady-State Voltammograms

Redox System	Type of Microelectrode	$r_{calculated}$[a]/μm
10 mM ferrocyanide with 1 M NaF in water	50 μm diameter platinum microelectrode	27.5
10 mM ferrocyanide with 1 M NaF in water	10 μm diameter platinum microelectrode	5.1
10 mM ferrocyanide with 1 M NaF in water	12.5 μm diameter gold microelectrode[b]	7.1
10 mM ferrocyanide with 1 M NaF in water	40 μm diameter gold microelectrode[c]	21.1
1 mM ferrocene with 0.1 M TBAFB in acetonitrile	40 μm diameter gold microelectrode[c]	29.1
10 mM ferrocyanide with 1 M NaF in water	12.5 μm diameter gold microelectrode[b]	6.5
10 mM ferrocyanide with 1 M NaF in water	40 μm diameter gold microelectrode[c]	20.5
1 mM ferrocene with 0.1 M TBAFB in acetonitrile	40 μm diameter gold microelectrode[c]	22.1

[a] These data are from the experiments conducted by ~50 students. The error values are ±1%. [b,c] Corresponds to different electrodes fabricated using wire of identical radius.

where i_{lim} is the steady-state limiting current (A), F is Faraday constant (96,500 C), and r is electrode radius (cm). By measuring the limiting current from steady-state sigmoidal-shaped voltammogram, r can be determined, provided the values of C^* and D are known. Sigmoidal-shaped voltammograms were observed in all the experiments indicating microelectrode characteristics. The cyclic voltammograms were reproducible even after 24 h in the solution, indicating good metal−glass sealing without any leakage (for example, a 10 μm diameter platinum microelectrode exhibited almost the same steady-state current value of 18 nA for the ferrocyanide redox system even after 24 h in the same solution). The cyclic voltammograms for 10 mM ferrocyanide with 1 M NaF with 40 μm Au and 10 μm Pt microdisk electrodes are shown in Figure 4. The radius of microelectrode was determined using eq 5. In determining r values, the following diffusion coefficient values (D) from the literature at 25 °C[10−12] were used.

$$D_{ferrocyanide/water} = 9.2 \times 10^{-6}\ cm^2/s$$

$$D_{ferrocene/acetonitrile} = 2.4 \times 10^{-5}\ cm^2/s$$

The calculated r values for different microelectrodes obtained from the steady-state voltammograms are shown in Table 1. The difference between the measured radius of the fabricated microelectrodes and radius of the commercial microelectrodes provided by the manufacturer is between 1 and 2%. The difference of measured radius for two different electrodes fabricated using wire of identical radius was not so significant (Table 1). This observation suggests that the microelectrodes fabricated by this technique are reproducible. The electrodes were shown to have similar characteristics to their commercial counterparts.

■ CONCLUSIONS

A microelectrode-based voltammetric technique is described for the undergraduate chemistry curriculum. The homemade potentiostat as well as the fabricated microelectrodes enhance the undergraduate chemical education by introducing students to this important technique. This homemade potentiostat as well as the microelectrodes were successfully operated in the laboratory. By setting up a low-cost homemade microelectrode and potentiostat, this important electroanalytical technique became more accessible to students at the undergraduate level. Fabricated gold microelectrodes can be used to study the barrier properties of alkanethiol SAM as shown in the Supporting Information. Finally, it is important to mention here that Sur et al.[13] had studied the in situ microwave activation of electrochemical processes by self-focusing intense microwave radiation into a region close to electrode−electrolyte interface by placing metal microelectrodes inside a microwave cavity. They also demonstrated extreme faradaic current enhancement up to 3 orders of magnitude for various redox systems using a 25 μm Pt microelectrode, which can act as an "antenna" to enhance the microwave effect considerably in the vicinity of microelectrode surface.

■ ASSOCIATED CONTENT

Supporting Information

Instructions for the students; data from fabricated gold microelectrodes studying the barrier properties of alkanethiol SAM. This material is available via the Internet at http://pubs.acs.org.

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(9) Op Amp CA 3140 for the control amplifier and voltage follower and AD 515 (Analog Devices) as current amplifier can be used for the fabrication of the potentiostat. AD 515 is a low-noise amplifier, having ultra low bias current of 75 fA, with low offset and thermal drift, and is ideal for measuring very low currents. However, measurement of such low currents is beset with the problem of line frequency (50 Hz) and its harmonic noise. Excellent signal-to-noise ratio can be obtained by using

an optional unity gain sallen key type low-pass filter having a cutoff frequency of 1 Hz. Low noise, low-drift Op Amp AD 743 (Analog Devices) can be used for fabricating the low pass filter.

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TECHNOLOGY REPORT

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Zotero: A Reference Manager for Everyone

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ABSTRACT: A review of Zotero 3.0b2, an open-source, cross-platform, all-in-one reference manager, document archiver, citation manager, and collaboration tool is presented. This report will discuss features such as collecting references, annotating references, integration with Microsoft Word and Open Office, backing up and synchronizing references, and sharing references with students and collaborators. Some useful but less commonly known tips and tricks are also described.

KEYWORDS: Continuing Education, First-Year Undergraduate/General, Graduate Education/Research, High School/Introductory Chemistry, Second-Year Undergraduate, Upper-Division Undergraduate, Interdisciplinary/Multidisciplinary, Internet/Web-Based Learning, Textbooks/Reference Books

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References can be shared with colleagues through public or private shared libraries. RSS newsfeeds can also be created for public group libraries. The ability to share references, annotations, and notes with colleagues makes for an effective collaborative tool. Other free reference managers worth considering include Mendeley[8] and Qiqqa.[9]

■ ASSOCIATED CONTENT

Supporting Information

Comparison of Zotero, Mendeley, Qiqqa, RefWorks, and EndNote. This material is available via the Internet at http://pubs.acs.org.

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COMMUNICATION

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Understanding Fischer Projection and Angular Line Representation Conversion

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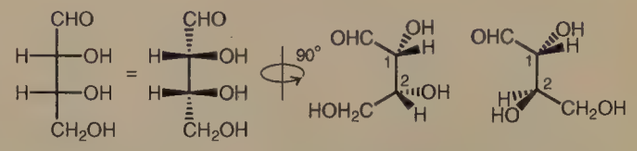
ABSTRACT: Difficulty for undergraduate students taking a basic organic chemistry course arises when they have to understand the relationship between the different molecular representations, for example, between Fischer and angular line representations. It is well known that some techniques describe the conversion between the Fisher and Howarth projections and others describe the conversion between the Fischer and angular line projections. This text offers an easy way to understand the conversion between the Fischer projection and angular line representation, emphasizing the structural relationship of the two models.



KEYWORDS: Second-Year Undergraduate, Organic Chemistry, Analogies/Transfer, Chirality/Optical Activity, Conformational Analysis

Difficulty for undergraduate students taking a basic organic chemistry course arises when they have to understand the relationship between the different molecular representations, for example, between Fischer and angular line representations. Some techniques to describe the conversion between the Fisher and Haworth projections have been published,[1−4] and other techniques to describe the conversion between the Fischer and angular line projections have been published as well.[5−8] This text offers an easy way to understand the conversion between the Fischer projection and angular line representation, emphasizing the structural relationship of the two models.

Fischer projections are widely used in the representation of systems with a large number of chiral centers. A simplified carbohydrate model with a single chiral center (**A**) is used to describe a method to understand the conversion. The method involves a 90° rotation of a Fischer projection to the right, around a vertical axis in the plane of the page (**B**), followed by a second 90° rotation in a counterclockwise direction around an axis perpendicular to the plane of the page, thus, converting a Fischer projection into an angular line projection (**C**). Figure 1 shows the conversion and could easily be used in conjunction with hand-held molecular models to further illustrate the conversion.

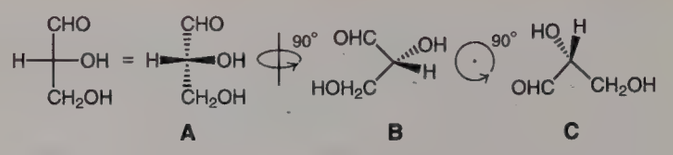


Figure 1. Conversion between Fischer projection and angular line representation for a compound with one chiral center.

An interesting feature in a Fischer projection of a molecule that contains multiple chiral centers (**D**) is that all the carbons in the chain acquire a completely eclipsed conformation (Figure 2). A 90° rotation to the right around a vertical axis in the plane of the page (**E**) illustrates this point. The Fischer projection is an angular line, flat, cyclic representation, where all the main chain carbons are completely eclipsed, this being the highest-energy conformer. The angular line conformation (**F**) results in the lowest-energy representation, where all the main chain carbons are in a staggered conformation in the classical representation of a zigzag.

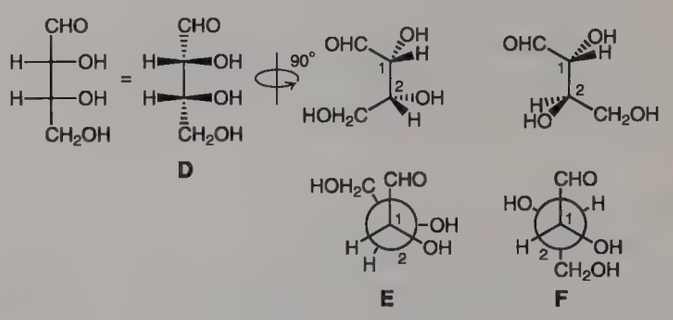


Figure 2. Conversion between Fischer projection and angular line representation for a compound with two chiral centers; eclipsed and anti Newman projections are shown below the angular line representations.

The general instructions to convert from a Fischer representation to the angular line representation are as follows:

- The Fischer projection performs a 90° rotation on a vertical axis in the plane of the page. If the rotation is done to the right, all the substituents located on the left side out of the plane of the page are now located on bold wedges out of the plane, and the substituents located on the right side entering the plane of the page are on dashed-wedges. The carbon chain stays in the plane similar to open cyclic representation (compare **D** and **E** in Figure 2).
- Draw a zigzag chain of an equal number of carbon atom to the central chain of the Fischer projection and the spatial relationship of the two substituents on the first chiral carbon will be the same as when the Fischer projection was rotated.

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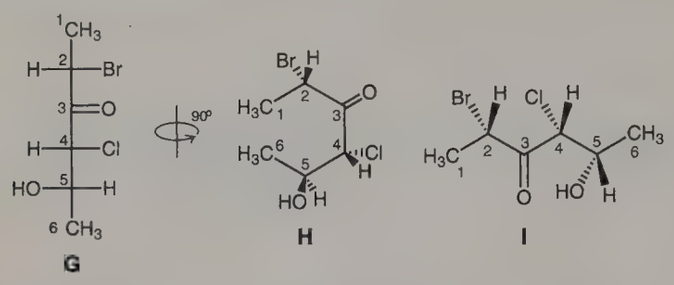

Figure 3. The compound (2*S*,4*R*,5*S*)-2-bromo-4-chloro-5-hydroxy-3-hexanone; conversion between Fischer projection and angular line representation.

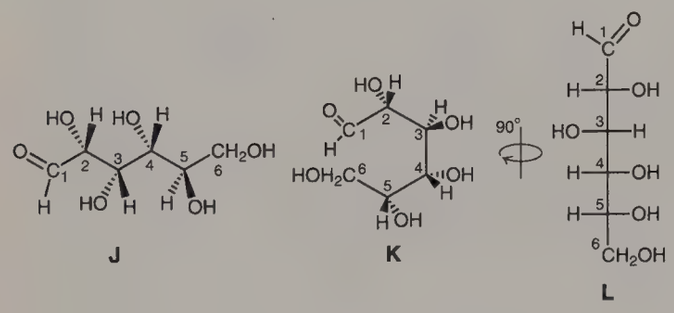

Figure 4. Conversion between angular line representation and Fischer projection for glucose.

The position of other substituents depends on whether they are on the same side or the opposite side in the Fischer projection (compare **E** and **F** in Figure 2).

The following guidelines help identify the type of spatial relationship between the substituents upon the conversion:

- The relationship between substituents on adjacent atoms in a Fischer projection will be reversed in the angular line representation.
- The relationship between two substituents on atoms separated by one atom in a Fischer projection will be the same in the angular line representation.

■ EXAMPLES

In (2*S*,4*R*,5*S*)-2-bromo-4-chloro-5-hydroxy-3-hexanone (Figure 3), C2 has a bromide substituent on the right side in the Fischer projection (**G**). This group is represented as a dashed bond behind the plane in angular line form (**H**). Note that the carbonyl group is located in the plane perpendicular to the page in the Fischer projection, shown in **G** on the right side. They are shown in the rotated Fischer projection and open-chain cyclic structure (**H**); the chlorine and bromine are located in the same side in the Fischer projection, and because they are separated from one another by the carbonyl carbon, the chlorine and bromine will end up on the same side in the zigzag angular line drawing (**I**) as dashed bonds behind the plane. Finally, the relationship between substituents chlorine and hydroxyl on adjacent atoms C4 and C5, respectively, in a Fischer projection will be reversed in the zigzag angular line representation.

As a final example of using this method, the representation of the conversion for glucose (Figure 4) begins by drawing an open cyclic chain (**K**) of an equal number of carbon atoms to the zigzag angular line representation (**J**). In **J**, C2 has a hydroxyl group represented as a dashed bond behind the plane and must remain the same in **K**; C3 has a hydroxyl group as a dashed bond too, and it would be reversed in a Fischer projection (**L**). C4 has a hydroxyl group that is separated from C2 by C3, then the relationship between the two hydroxyl groups will be the same in a Fischer projection; likewise. the relationship between the substituents on C4 and C5 will be reversed in a Fischer projection.

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ADDITION/CORRECTION

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Correction to An Introduction to Enzyme Kinetics, Part Deux

Addison Ault*

Journal of Chemical Education **2011**, *88*, 63. DOI: 10.1021/ed1004432

The originally submitted version of the article, "Representing Rate Equations for Enzyme-Catalyzed Reactions", was published in the January 2011 issue, instead of the accepted version, "An Introduction to Enzyme Kinetics, Part Deux". The Online version was corrected on December 16, 2011. The submitted version that appeared in the January 2011 issue is included as Supporting Information for reference. The author regrets the error.

■ ASSOCIATED CONTENT

Supporting Information

The originally submitted version of the article, "Representing Rate Equations for Enzyme-Catalyzed Reactions" as it appeared in print. This material is available via the Internet at http://pubs.acs.org.

Published: December 16, 2011

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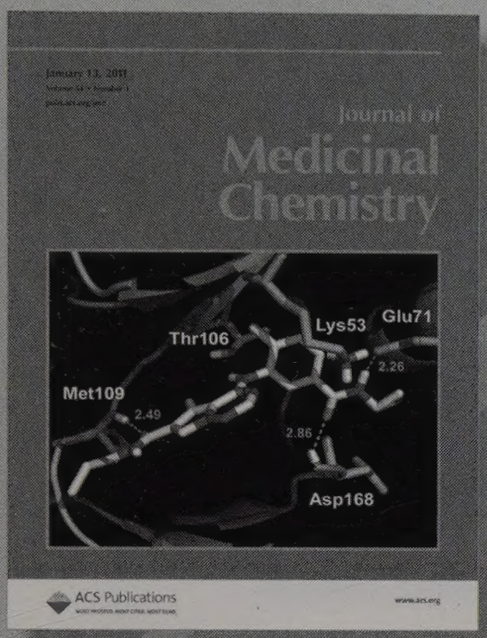

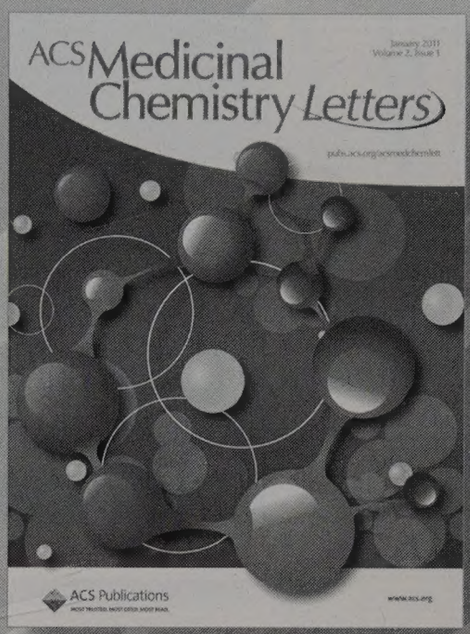

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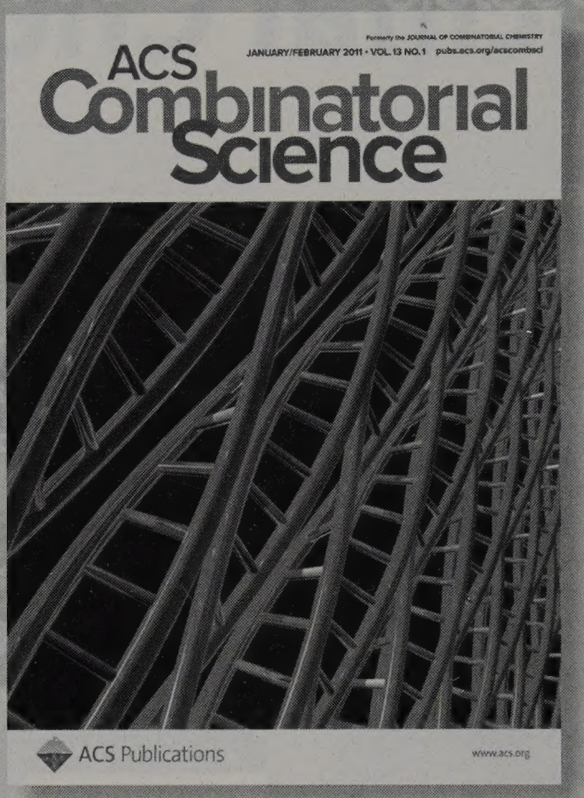

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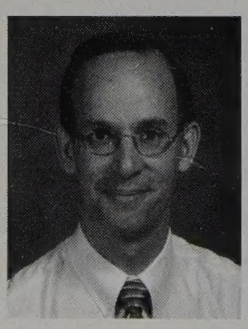

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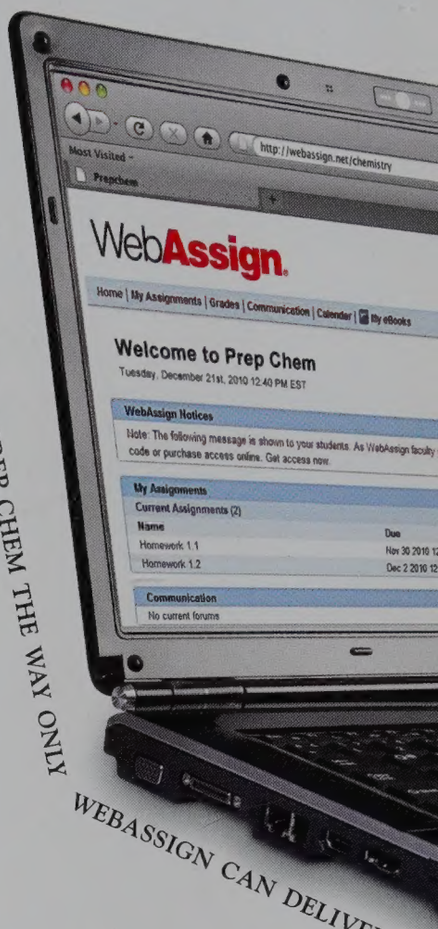

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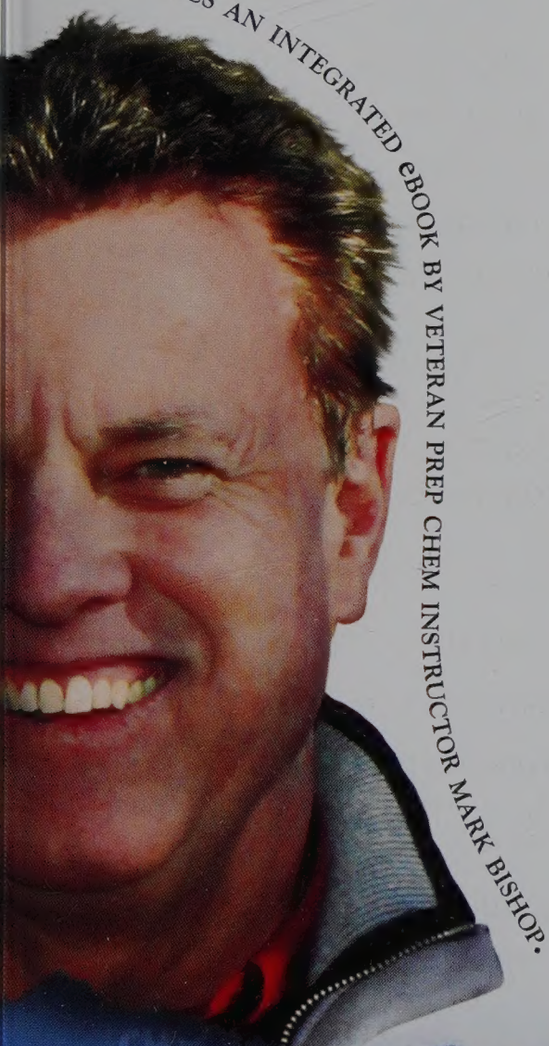

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